ABSTRACT

In this study, a supercritical fluid CO_2 (SF-CO_2) extraction and subcritical water extraction (SWE) were developed with the aim of overcoming the disadvantages associated with the conventional soxhlet extraction. The SFE conditions utilised extraction temperature of 60 ^oC, extraction pressure of 200 bar, 15% methanol as co-solvent, extraction flow rate at 2.0 mL/min, 30 minute extraction and *n*-hexane as the analyte trapping solvent for PCBs extraction from sludge. A lab-made SWE dynamic extraction system was successfully developed for the dynamic SWE of PCBs from sludge samples. For the SWE, the optimized conditions employed an extraction temperature of 250 ^oC, extraction pressure of 50 bar, 1 min static followed by 10 min dynamic extraction and analyte collection in dichloromethane. Both the SF-CO_2 extraction and SWE showed good PCB recoveries (50 to 99 %) compared to soxhlet extraction (52 to 75 % PCB recoveries). From the two extraction methods investigated, it was clear that the SF-CO_2 technique was better in terms of recovery, good reproducibility and speed of analysis compared to the SWE method and it was therefore chosen as the method of choice for the analysis of real sludge samples. The PCBs under study (PCB 28, 52, 101, 118, 138, 153 and 180) when analysed using GC-ECD gave low detection limits in the range of 0.016 to 0.048 μg/mL. The concentration of PCBs in sludge samples extracted using SF-CO_2 were in the range of 31.2 to 82.0 μg/kg as compared to that using soxhlet with PCB levels from 20.2 to 60.1 μg/kg. Method development for analysis of PAHs in sewage sludge was also carried out using spiked samples and real samples. Extraction of PAHs in sewage sludge was utilised using Soxhlet extraction followed by extract clean-up procedure. Limits of detection for five PAHs (naphthalene, fluoroanthene, phenanthrene, benzo(a)fluoroanthene and benzo(a)pyrene) were in the range of 5 mg/L to 20 mg/L. Analysis of OCP (lindane, dieldrin, TDE, pp-DDT and heptachlor) in sewage sludge was also successfully developed. The use of GC-ECD instrument was successful in separating and detecting all OCPs under study. In this study, limits of detection of OCP were in the range of 7.8 to 39.0 μg/kg. Extracts of OCP in sewage sludge were prepared using Soxhlet extraction followed by clean-up procedure using silica column. The concentrations of OCPs were in the range of (47 to 159 μg/kg) in sewage sludge. The characterization of sludge samples from other aspects was also carried out. The sludge samples showed high water content (37 to 90 %) with slightly acidic pH values ranging from 4.3 to 6.6. All samples were found to be chemically stable and the percentages of nutrient (TKN, P and K) were in the range of 0.059 to 0.98 %, 0.05 to 0.15 % and 0.12 to 0.16 %, respectively. Heavy metal studies showed that metal content in municipal sewage sludge samples were below the USEPA limit.

ABSTRAK

Dalam kajian ini, pengekstrakan lampau genting karbon dioksida (SF-CO_2) dan pengekstrakan sub lampau genting air (SWE) telah dibangunkan dengan tujuan untuk mengatasi kelemahan pengekstrakan soxhlet yang konvensional. Parameter pengekstrakan SF-CO_2 melibatkan suhu pengekstrakan pada 60 ^{o}C, tekanan pengekstrakan pada 200 bar, 15 % metanol sebagai kedua pelarut, kadar alir 2.0 mL/min, 30 minit masa pengekstrakan dan *n*-heksana digunakan sebagai perangkap analit bagi pengekstrakan PCB di dalam enapcemar. Satu sistem pengekstrakan dinamik SWE telah berjaya dibangunkan di makmal. Bagi SWE, parameter yang dipilih untuk pengekstrakan PCB melibatkan suhu pengekstrakan pada 250 ^{o}C, tekanan pengekstrakan pada 50 bar, 1 minit pengekstrakan statik diikuti dengan 10 minit pengekstrakan dinamik dan analit dikumpul dalam pelarut diklorometana. Kedua-dua kaedah SF-CO_2 pengekstrakan dan SWE menunjukkan peratus pengembalian PCB yang baik (50 hingga 99 %) berbanding pengekstrakan soxhlet (52 hingga 75 %). Daripada dua pengekstrakan yang dikaji, kaedah pengekstrakan SF-CO_2 adalah yang lebih baik berdasarkan peratusan pengembalian, kebolehulangan yang baik serta masa analisis berbanding dengan pengekstrakan SWE dan seterusnya digunakan bagi analisis kandungan PCB di dalam sampel sebenar enapcemar. Had pengesanan PCB yang dikaji (PCB 28, 52, 101, 118, 138 153 dan 180) adalah rendah dalam julat di antara 0.016 hingga 0.048 μg/mL. Kepekatan PCB di dalam enapcemar menggunakan pengekstrakan SF-CO_2 adalah di antara 31.2 hingga 82.0 μg/kg berbanding dengan pengekstrakan soxhlet yang memberikan aras PCB antara 20.2 hingga 60.1 μg/kg. Pembangunan kaedah untuk analisis PAH di dalam enapcemar juga dikaji menggunakan sampel pakuan dan sampel sebenar. Pengekstrakan PAH di dalam enapcemar menggunakan pengekstrakan soxhlet diikuti kaedah pembersihan hasil ekstrak. Had pengesanan untuk lima PAH (naftalena, fluoroantena, phenantrena, benzo(a)fluoroantena dan benzo(a)pyrena) dalam julat 5 mg/L hingga 20 mg/L. Analisis OCP (lindana, dieldrin, TDE, pp-DDT and heptaklor) juga berjaya dibangunkan. Instrumen GC-ECD berjaya memisahkan dan mengesan semua OCP yang dikaji. Di dalam kajian ini, had pengesanan OCP dalam julat 7.8 hingga 39.0 μg/kg. Ekstrak OCP di dalam enapcemar menggunakan pengekstrakan soxhlet diikuti pembersihan menggunakan turus silika. Kepekatan OCP adalah 47 hingga 159 μg/kg di dalam sampel enapcemar. Pencirian sampel enapcemar dari aspek lain juga dijalankan. Sampel enapcemar menunjukkan kandungan air yang tinggi (37 hingga 90 %) dan nilai pH didapati sedikit berasid (julat 4.3 hingga 6.6). Semua sampel enapcemar adalah stabil dari segi kimia dengan peratus nutrien (TKN, P dan K) adalah masing-masing 0.059 hingga 0.98 %, 0.05 hingga 0.15 % dan 0.12 hingga 0.16 %. Kajian logam berat menunjukkan kandungan logam adalah di bawah tahap USEPA.

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Bp	-	Biphenyls
CE	-	Capillary electrophoresis
CO_2	-	Carbon dioxide
DCM	-	Dichloromethane
ECD	-	Electron Capture Detector
GC	-	Gas Chromatography
HPLC	-	High Performance Liquid Chromatography
IS	-	Internal Standard
IWK	-	Indah Water Konsortium
LOD	-	Limit of Detection
mg/kg	-	milligram per kilogram
mL	-	millilitre
mL/min	-	millilitre per minute
MeOH	-	Methanol
^oC	-	Celsius degree
ODS	-	Octadecylsilane
PCBs	-	Polychlorinated Biphenyls
PE	-	Population Equivalent
ppm	-	Part per million
RSD	-	Relative Standard Deviation
SF CO_2	-	Supercritical Fluid Carbon Dioxide
SFE	-	Supercritical Fluid Extraction
SPE	-	Solid Phase Extraction
SWE	-	Subcritical Water Extraction

TKN	-	Total Kjeldahl Nitrogen
t_r	-	Retention Time
USEPA	-	United State Environmental Protection Agency
WTP	-	Wastewater Treatment Plant
ε	-	Dielectric Constant
μL	-	microlitre
%	-	Percent
Pc	-	Critical Pressure
Tc	-	Critical Temperature
Tp	-	Triple point
Cp	-	Critical point
OCP	-	Organochlorine Pesticides
PAH	-	Polycyclic Aromatic Hydrocarbon

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CHAPTER 1

INTRODUCTION

1.1 Municipal Sewage Sludge

Since 1994, sludge has become a major issue in Malaysia. The responsibility for the provision of sewerage services in Malaysia is vested in the Federal Government through the Sewerage Services Department (SSD), which was established in 1994 under the Ministry of Housing and Local Government. To date, most of our river systems are polluted with domestic sewage. It has been reported that over 70 percent of the rivers in Malaysia are classified as polluted and the major source has been identified as human waste. The problem posed a major threat to our water resources (Velayutham and Kadir, 1999).

Municipal sewage sludge is the product of the municipal wastewater treatment plant (Figure 1.1) whereby it consists of the final accumulation of sewage sludges from urban wastewater treatment plants from agriculture, industries and also domestic waste. Its contents is mainly water (approximately 95 %), plus the presence of other substances, such as insoluble and soluble organic matter, nutrients, microorganisms, pathogens, metals, soluble salts, and other minerals (Torrey, 1979). The composition varies due to its function of the infrastructures of the respective residential areas, weather conditions and so on.

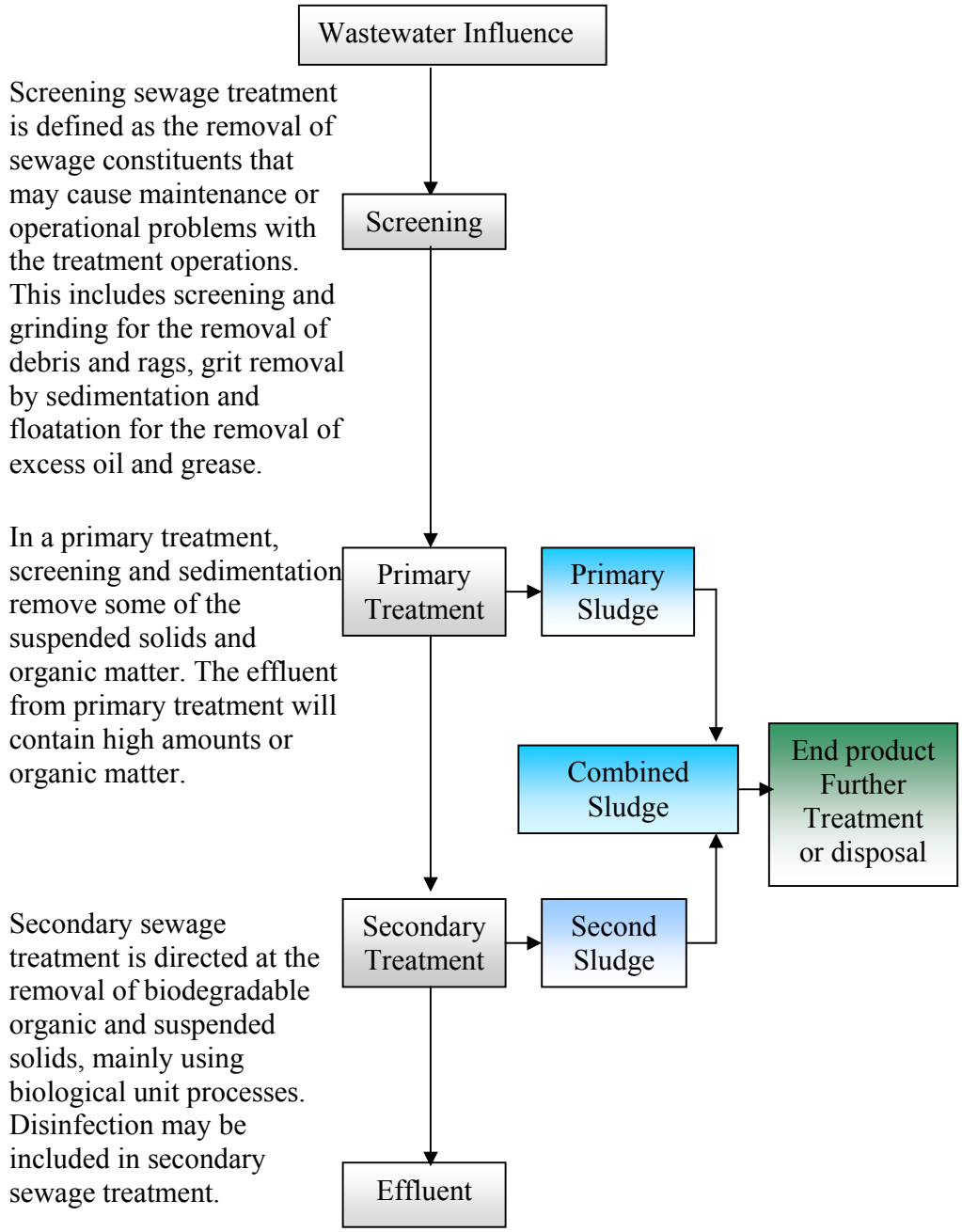


Figure 1.1 Flow chart of basic wastewater treatment process

In an attempt to accelerate the upgrading of sewerage services in the urban areas, the Government signed a 28-year concession agreement with the private sector Indah

Water Konsortium (IWK) in April 1994, thereby effectively privatizing the operation and maintenance of sewerage services, and including under the agreement a capital works programmer to expand the sewerage coverage to about 85% of the population in the major towns and about 30% of the population in the smaller towns by 2022. The concession agreement also covered septic tank desludging. By the end of 2001, sewerage services in 84 local authorities had been taken over by IWK, excluding the states of Sabah and Sarawak in East Malaysia, but covering all Peninsular Malaysia with the exception of Kelantan State and the municipality of Johor Bahru (Bradley and Dhanagunan, 2004).

The number of activated sludge facilities in Peninsular Malaysia is now 1683, having increased almost twenty-fold since the services were privatized in 1994. The increase in coverage of sewerage services has been fuelled to a great extent by the rapid rate of economic development that has occurred in Peninsular Malaysia over the last 15 years, the rate of urbanization and industrialization being particularly high in the Federal Territory of Kuala Lumpur and the adjacent Klang Valley region in Selangor State, as well as in the northern state of Penang (Bradley and Dhanagunan, 2004).

However, this practice has the potential to create an environmental pollution problem since the heavy metal contents (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn), toxic organic compounds and pathogenic organisms are still in the sewage sludge (EU, 2000). Pollution problems may arise if toxics are mobilized into the soil solution and are either taken up by plants or transported in drainage waters to associate water supplies. The pollutant may then enter the human food chain through the consumption of such plants or through intake of contaminated waters. For sewage sludge and compost, the limit values for heavy metal have been established as the quality criteria (US EPA, 1984). Nevertheless, the characterization and long term observation of organic contaminants in sludge have received little attention so far.

1.2 Criteria in Re-use and Disposal of Sludge

Certain regulations have been made regarding the re-use and disposal of sewage sludge. Some of the more desirable materials in sludge also need to be monitored for the most beneficial reuse of the sludge. These parameters include:

i. Water and plant nutrients (nitrogen, phosphorus and potassium)
ii. Organic matter and non-toxic trace elements
iii. Microorganisms

Furthermore, parameters such as organic content, toxic organics, nutrients, pathogens and hazardous metals have to be taken into account if they will be distributed for re-use (US EPA, 1984). Some of these hazardous elements are:

i. Potentially toxic elements (eg: zinc, copper, nickel, cadmium, lead, mercury, chromium, arsenic and selenium)
ii. Organic micro-pollutants (eg: Linear Alkylbenzene Sulphonated (LAS), Di (2-ethylhexy) phthalate (DEHP), Nonylphenole (NP), Polychlorinated Biphenyls (PCBs) and others)
iii. Pathogenic organisms

The above mentioned parameters, together with other important considerations need a careful attention when deciding on the best alternative for the reuse or disposal of sewage sludge.

1.3 Beneficial Uses of Sludge

The beneficial usage of sludge are they can be recycled soil for the application in farming practices, horticulture, land restoration and reclamation, landfill cover, forestry energy value and product.

1.3.1 Farming Practices

The agriculture use of sludge is linked to the fertilizing value of nitrogen and phosphorus. About 1 to 5% of dry matter is phosphorous and nitrogen each (Lasa *et al.,* 1997). Other compounds present in sludge of agriculture value are potassium, sulphur, magnesium, sodium and elements such as like boron, cobalt, and selenium.

Sewage sludge can improve soil quality (nutrients, pH balance, trace elements), give better physical characteristics (improves organic matter, water holding capacity, irrigation, stability and workability) and enhances biological activity (greater water retention and aeration stimulating root growth, increases worm and micro-organisms populations). The net effect is improved soil quality and agricultural yields.

1.3.2 Horticulture

Sludge can be thermally processed or composted using crop residues or municipal solid wastes, green or wood processing wastes. The products are aesthetically acceptable and suitable for soil conditioning and fertilizer applications in situations where a direct sludge application might not be acceptable or practical, such as in garden, public parks and highway verges (Stabnikova *et al.,* 2005). Even in Malaysia, the waste sludge was mixed with compost to cut the costs for gardening.

1.3.3 Land Restoration and Reclamation

The surface of direct and disturbed land is often deficient in organic matter, nitrogen and phosphorus (Speir *et al*., 2003). On some sites, where nutrient soil may not be available, cover materials are needed which can then be converted into suitable

topsoil for sustaining plant growth. Sewage sludge contains the organic matter and fertilizer value needed to provide a stable medium for the site and also to help plant growth (Mantovi *et al*., 2004).

1.3.4 Landfill Cover

Sewage sludge can be used as daily and final cover for landfill sites, providing a consistent blanket that serves to reduce nuisance during on going operation and ultimately restore the filled site for a subsequent beneficial use (Skousen and Clinger, 1986; Hing *et al*., 1998).

1.3.5 Forestry

Some soils are more suitable for developing woodlands, especially in producing energy crops and wood products. Sludge improves tree growth by providing the appropriate nutrients such as the use of sludge could be very beneficial to fertilize or improve forestry soils (Marx *et al.,* 1995; Hing *et al*., 1998).

1.3.6 Energy Value

Sludge has an organic content that can be transformed into or used as fuel. A combination of technologies such as digestion, drying and incineration makes this possible (Hing *et al*., 1998). By sludge digestion, biogas is produced that consists normally of methane, which can be used on site for other process purposes such as heating, filtration and onwards. On other occasions, it is converted into electricity

distribution through national grids. Biogas can also be converted into a renewable fuel and be used by Natural Gas Vehicles (NGV) buses, trucks and cars, which is adapted from fossil fuel (Wang *et al*., 1997).

1.3.7 Alternative Product

The physical and chemical properties have made sewage sludge suitable for a range of alternative products, although most of these are currently not economically viable. A number of products could be derived directly from sludge or as an admixture with other material. These include building materials such as bricks and cement (Hing *et al*., 1998; Suzuki and Tanaka, 1997). Sludge can also be used as material for producing the watertight top layer of a landfill. Ash from incineration is a potential source of phosphorus for fertilizer manufacture or a potential building material such as blocks and aggregate or as a raw material in cement industry (Favoni *et al*., 2005).

1.4 Organic Sludge Contaminants

The presence of organic micro pollutants such as Polychlorinated Biphenyls in municipal sewage sludge is a major problem on account of risks associated with the agricultural use of the sludge. Sewage sludge contains many other organic contaminants such as organic halogen compounds (AOX), Linear Alkylbenzene Sulphonated (LAS), Di (2-ethylhexy) phthalate (DEHP), Nonylphenole (NP) and Nonylphenole Ethoxylates (NPE) with 1 or 2 ethoxy groups, Polynuclear Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs) and Organochlorine (OCP) (Abad *et al.,* 2005). PCBs, PAH and OCP are the major contaminants frequently found in sewage sludge (Gibson *et al.,* 2005).

1.4.1 Polychlorinated Biphenyls

PCBs are organic chemicals with characteristics similar to that of Dichlorodiphenyltrichloroethane (DDT). They are produced commercially by catalytic chlorination of biphenyls producing a complex mixture of multiple isomers with different degrees of chlorination yielding up to 209 products called congeners (Hutzinger *et al.,* 1974). PCB congeners with the same number of chlorine atoms are known as homologues, and the homologues with different chlorine positions are called isomers (Erickson, 1992). As a result of the widespread application and their stabilities, PCB has since become a major environmental concern worldwide.

PCBs are made up of a biphenyl nucleus with 1–10 chlorine atoms having a chemical formula of $C_{12}H_{10-n}Cl_n$. The basic structure of PCB is shown in Figure 1.2. During PCBs manufacturing process, a mixture of compounds with molecular weight ranging from 188 to 439.7 g/mol is produced depending on the number of chlorine atoms attached to the biphenyl ring. Toxic congeners carry between 5 to 10 chlorine atoms, mostly in *para*- and *meta*-positions, however, the congener substituted at the 3,4-*ortho* positions are considered the most toxic (Hutzinger *et al*., 1974).

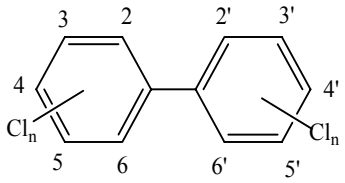


Figure 1.2 General structural formula of PCBs

PCBs are poorly soluble in water but extremely soluble in oils and fats. Their solubility in water decreases with the increase in the degree of chlorination. The solubility ranges from 6 ppm to 0.007 ppm for monochlorobiphenyl and octachlorobiphenyl respectively. Although decachlorobiphenyl has higher chlorine content, its solubility is twice that of octachlorobiphenyl. The solubility also varies among congeners which have the same number of chlorine atoms (Erickson, 1992).

PCBs are marketed with respect to the percentage of their chlorine content (by weight) and are available under several trade names, for example Clophen (Bayer, Germany), Aroclor (Monsanto, USA), Kanechlor (Kanegafuchi, Japan), Santotherm (Mitsubishi, Japan), Phenoclor and Pyralene (Prodolec, France) (Hutzinger *et al*., 1974). Table 1.1 shows the PCB homologues according to IUPAC and their chlorine position atom in the biphenyl ring. Only seven out of the 209 PCBs listed as priority pollutants by the USEPA were considered in this study due to their common occurrence in sewage sludge. The target PCBs were PCB No 28, 52, 101, 118, 138, 153 and 180**.**

1.4.1.1 Uses of PCBs

For several decades, PCBs were used extensively in a wide range of industrial applications such as oil in transformers, dielectrics in capacitors, hydraulic fluids in hydraulic tools and equipment and heat exchange liquids. PCBs were also used as lubricants for turbines and pumps, in the formulation of cutting oils for metal treatment, and, to a lesser extent, in applications such as plasticizers, surface coatings, adhesives, pesticides, carbonless copy paper, inks, dyes, and waxes (Hutzinger *et al*., 1974).

Table 1.1 Nomenclature of PCB homologues according to IUPAC

PCB no	Structure	PCB no	Structure
Monochlorobiphenyls		Tetrachlorobiphenyls	
1	2	40	2,2',3,3'
2	3	41	2,2',3,4
3	4	42	2,2',3,4'
		43	2,2',3,5
Dichlorobiphenyls		44	2,2,3,5'
4	2,2’	45	2,2',3,6
5	2,3	46	2,2',3,6'
6	2,3’	47	2,2’,4,4’
7	2,4	48	2,2',4,5
8	2,4'	49	2,2',4,5'
9	2.5	50	2,2',4,6
10	2,6	51	2,2',4,6'
11	3,3'	52	2,2',5,5'
12	3,4	53	2,2',5,6'
13	3,4'	54	2,2',6,6'
14	3,5	55	2,3,3',4
15	4,4'	56	2,3,3',4'
		57	2,3,3',5
Trichlorobiphenyls		58	2,3,3',5'
16	2,2',3	59	2,3,3',6
17	2,2',4	60	2,3,4,4'
18	2,2',5	61	2,3,4,5
19	2,2’,6	62	2,3,4,6
20	2,3,3'	63	2,3,4',5
21	2,3,4	64	2,3,4',6
22	2,3,4'	65	2,3,5,6
23	2,3,5	66	2,3',4,4'
24	2,3,6	67	2,3',4,5
25	2,3',4	68	2,3',4,5'
26	2,3',5	69	2,3',4,6
27	2,3',6	70	2,3',4',5
28	2,4,4'	71	2,3,4',6
29	2,4,5	72	2,3',5,5'
30	2,4,6	73	2,3',5',6
31	2,4',5	74	2,4,4',5
32	2,4',6	75	2,4,4',6
33	2',3,4	76	2',3,4,5
34	2',3,5	77	3,3',4,4'
35	3,3',4	78	3,3',4,5
36	3,3',5	79	3,3',4,5'
37	3,4,4'	80	3,3',5,5'
38	3,4,5	81	3,4,4',5
39	3,4',5		

Table 1.1 continued

PCB no	Structure	PCB no	Structure
Pentachlorobiphenyls		124	2',3,4,5,5'
82	2,2',3,3',4	125	2',3,4,5,6'
83	2,2',3,3',5	126	3,3',4,4',5
84	2,2',3,3',6	127	3,3',4,5,5'
85	2,2',3,4,4'		
86	2,2',3,4,5	Hexachlorobiphenyls	
87	2,2',3,4,5'	128	2,2',3,3',4,4'
88	2,2',3,4,6	129	2,2',3,3',4,5
89	2,2',3,4,6'	130	2,2',3,3',4,5'
90	2,2',3,4',5	131	2,2',3,3',4,6
91	2,2',3,4',6	132	2,2',3,3',4,6'
92	2,2',3,5,5'	133	2,2',3,3',5,5'
93	2,2',3,5,6	134	2,2',3,3',5,6
94	2,2',3,5,6'	135	2,2',3,3',5,6'
95	2,2',3,5',6	136	2,2',3,3',6,6'
96	2,2',3,6,6'	137	2,2',3,4,4',5
97	2,2',3',4,5	138	2,2,3,4,4',5'
98	2,2',3,4,6	139	2,2',3,4,4',6
99	2,2',4,4',5	140	2,2',3,4,4',6'
100	2,2',4,4',6	141	2,2',3,4,5,5'
101	2,2',4,5,5'	142	2,2',3,4,5,6
102	2,2',4,5,6'	143	2,2',3,4,5,6'
103	2,2',4,5',6	144	2,2',3,4,5',6
104	2,2',4,6,6'	145	2,2',3,4,6,6'
105	2,3,3',4,4'	146	2,2',3,4',5,5'
106	2,3,3',4,5	147	2,2',3,4',5,6
107	2,3,3',4',5	148	2,2',3,4,5,6'
108	2,3,3',4,5'	149	2,2',3,4',5’6
109	2,3,3',4,6	150	2,2',3,4',6,6'
110	2,3,3',4',6	151	2,2',3,5,5',6
111	2,3,3',5,5'	152	2,2',3,5,6,6
112	2,3,3',5,6	153	2,2',4,4',5,5'
113	2,3,3',5',6	154	2,2',4,4,5,6'
114	2,3,4,4',5	155	2,2',4,4',6,6'
115	2,3,4,4',6	156	2,3,3',4,4',5
116	2,3,4,5,6	157	2,3,3',4,4',5'
117	2,3,4',5,6	158	2,3,3',4,4',6
118	2,3',4,4',5	159	2,3,3',4,5,5'
119	2,3,4,4',6	160	2,3,4',4,5,6
120	2,3',4,5,5'	161	2,3,3',4,5',6
121	2,3',4,5',6	162	2,3,3',4',5,5'
122	2',3,3',4,5	163	2,3,3',4',5,6
123	2',3,4,4',5	164	2,3,3',4',5',6

Table 1.1 continued

PCB no	Structure	PCB no	Structure
165	2,3,3',5,5',6	192	2,3,3',4,4',5,6
166	2,3,4,4',5,6	193	2,3,3',4,4',5',6
167	2,3',4,4',5,5'	189	2,3,3',4,5,5',6
168	2,3',4,4',5',6	190	2,3,3',4',5,5',6
169	3,3',4,4',5,5'		
		Octachlorobiphenyls	
Heptachlorobiphenyls		194	2,2',3,3',4,4',5,5'
170	2,2',3,3',4,4',5	195	2,2',3,3',4,4',5,6
171	2,2',3,3',4,4',6	196	2,2',3,3',4,4',5',6
172	2,2',3,3',4,5,5'	197	2,2',3,3',4,4',6,6'
173	2,2',3,3',4,5,6	198	2,2',3,3',4,5,5',6
174	2,2',3,3',4,5,6'	199	2,2,3,3',4',5,5',6
175	2,2',3,3',4,5',6	200	2,2',3,3',4,5,6,6'
176	2,2',3,3',4,6,6'	201	2,2',3,3',4,5',6,6'
177	2,2',3,3',4',5,6	202	2,2',3,3',5,5',6,6'
178	2,2',3,3',5,5',6	203	2,2',3,4,4',5,5',6
179	2,2',3,3',5,6,6'	204	2,2',3,4,4',5,6,6'
180	2,2',3,4,4',5,5'	205	2,3,3',4,4',5,5',6'
181	2,2',3,4,4',5,6		
182	2,2',3,4,4',5,6'	Nonachlorobiphenyls	
183	2,2',3,4,4',5',6	206	2,2',3,3',4,4',5,5',6
184	2,2',3,4,4',6,6'	207	2,2',3,3',4,4',5,6,6'
185	2,2',3,4,5,5',6	208	2,2',3,3',4,5,5',6,6'
186	2,2',3,4,5,6,6'		
187	2,2',3,4',5,5',6	Decachlorobiphenyl	
188	2,2',3,4',5,6,6'	209	2,2',3,3',4,4',5,5',6,6'
191	2,3,3',4,4',5,5'		

1.4.1.2 General Characteristics of Common PCBs

For determination and understanding of PCBs extractability, the most important parameters are PCBs solubility and boiling point (Hutzinger *et al.,* 1974). Some general characteristics of the PCBs according to the isomer group are listed below in Table 1.2.

Table 1.2 Physical properties of PCB homologues

PCB Isomer Group	Melting Point (ºC)	Boiling Point (ºC)	Vapour Pressure (Pa) at 25ºC	Water Solubility at 25ºC (g/m^3)
Biphenyl	71	256	4.9	9.3
Monochlorobiphenyl	25-77.9	285	1.1	4.0
Dichlorobiphenyl	24.4-149	312	0.24	1.6
Trichlorobiphenyl	28.87	337	0.054	0.65
Tetrachlorobiphenyl	47-180	360	0.012	0.26
Pentachlorobiphenyl	76.5-124	381	2.6×10^{-3}	0.099
Hexachlorobiphenyl	77-150	400	5.8×10^{-4}	0.038
Heptachlorobiphenyl	122.4-149	417	1.3×10^{-4}	0.014
Octachlorobiphenyl	159-162	432	2.8×10^{-5}	5.5×10^{-3}
Nonachlorobiphenyl	182.8-206	445	6.3×10^{-6}	2.0×10^{-3}
Decachlorobiphenyl	305.9	456	1.4×10^{-6}	7.6×10^{-4}

1.4.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a complex class of organic compounds containing two or more fused aromatic rings, and containing only carbon and hydrogen atoms. The physical and chemical properties of PAHs are determined by their conjugated pi-electron systems, which are dependent on the number of aromatic rings and the molecular mass. The smallest member of the PAH family is naphthalene, a two-ring compound, which is found in the vapour phase in the atmosphere. Three to five ring-PAHs can be found in both the vapour and particulate phases in air. PAHs consisting of five or more rings tend to be solids adsorbed onto other particulate matter in the atmosphere.

The term 'polycyclic aromatic compounds' or 'polycyclic organic matter' is used to include similar compounds with nitrogen, oxygen or sulfur substituents such as nitro-PAHs, hydroxy-PAHs and heterocyclic compounds. This report will concentrate only on compounds that fall in the category of polycyclic aromatic hydrocarbons. Sometimes the term 'polynuclear' is used in the literature instead of 'polycyclic' to describe these compounds.

Wastewater catchments receive PAHs from two main sources: industrial and domestic fossil fuel spillages and urban runoff inputs that flush the hydrocarbons deposited on the ground surface from vehicles or heating systems. As a result of their very low aqueous solubility, PAHs are efficiently removed from the water during sedimentation in the wastewater treatment process.

The 16 PAHs are naphthalene (Naph), acenaphthylene (Ac), acenaphthene (Ace), fluorine (Flu), phenanthrene (phe), anthracene (Anth), fluoroanthene (Fluo), pyrene (pyr), benzo[a]pyrene (BaPy), dibenzo[a,h]anthracene (DiAn), chrysene (Chr), benzo[b]fluoroanthene (BkFl), benzo[g,h,I]perylene (BePe) and indeno[1,2,3-cd]pyrene (InPy), have been selected by the US EPA as “Consent Decree” priority pollutants (Figure 1.1). These compounds typically contain two or more benzene rings. The carcinogenicity of the PAHs are suspected to arise when four or more benzene rings are present in the compound.

PAHs are released into the atmosphere as a complex mixture of compounds during incomplete combustion of organic matter. They can be emitted from wood burning heaters, agricultural waste burning, motor vehicle exhaust, cigarette smoke, asphalt road and roofing operations. PAHs are widespread contaminants of the environment and a number of them are either known or suspected carcinogens. Benzo(a)pyrene, a widely reported five-ring PAH, is known for its carcinogenic potency. Although hundreds of PAHs have been identified in atmospheric particles (Lao *et al*., 1973; Lee *et al*., 1976), toxicological endpoint and/or exposure data are available for only 33 PAHs.

PAHs enter the environment mostly as releases to air from volcanoes, forest fires, residential wood burning, and exhaust from automobiles and trucks. They can also enter surface water through discharges from industrial plants and waste water treatment plants, and they can be released to soils at hazardous waste sites if they escape from storage containers. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water, and how easily they evaporate into the air. PAHs in general do not easily dissolve in water. They are present in air as vapors or stuck to the surfaces of small solid particles. They can travel long distances before they return to earth in rainfall or particle settling. Some PAHs evaporate into the atmosphere from surface waters, but most stick to solid particles and settle to the bottoms of rivers or lakes. In soils, PAHs are most likely to stick tightly to particles. Some PAHs evaporate from surface soils to air. Certain PAHs in soils also contaminate underground water.

The PAH content of plants and animals living on the land or in water can be many times higher than the content of PAHs in soil or water. PAHs can break down to longer-lasting products by reacting with sunlight and other chemicals in the air, generally over a period of days to weeks. PAHs can enter the human body through the lungs during breathing (usually stuck to particles or dust).

PAHs can enter all the tissues of the body that contain fat. They tend to be stored mostly in your kidneys, liver, and fat. Smaller amounts are stored in the spleen, adrenal glands, and ovaries. PAHs are changed by all tissues in the body into many different substances. Some of these substances are more harmful and some are less harmful than the original PAHs. Results from animal studies show that PAHs do not tend to be stored in the human body for a long time. Most PAHs that enter the body leave within a few days, primarily in the feces and urine.

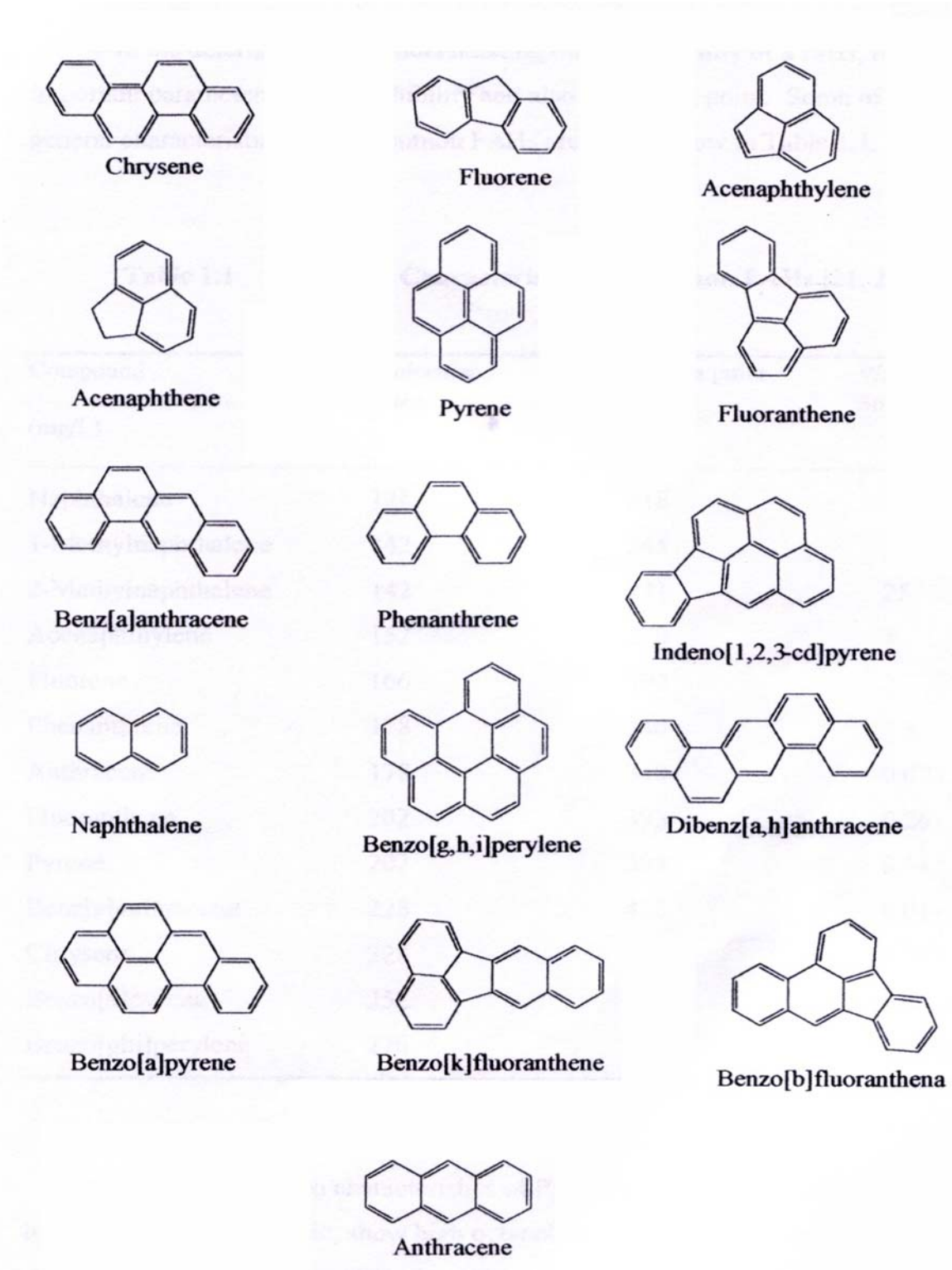


Figure 1.3 Structures of the 16 PAH Termed as Priority Pollutants by the United States Environmental Protection Agency

1.4.2.1 General Characteristics of Common PAHs

For determination and understanding of PAHs extractability, the most important parameters are PAHs solubility and boiling point. Some general characteristics of the PAHs according to the isomer group are listed below in Table 1.3.

Table 1.3 Physical properties of PAH homologues

Compound (mg/L)	Molecular Mass	Boiling Point (ºC)	Water Solubility at 25ºC (g/m^3)
Naphthalene	128	218	32
1-Methylnaphthalene	142	245	29
2-Methylnaphthalene	142	241	25
Acenaphthylene	152	270	4
Fluorene	166	297	2
Phenanthrene	178	340	1.3
Anthracene	178	340	0.073
Fluoranthene	202	393	0.26
Pyrene	202	394	0.14
Benzo[a]anthracene	228	438	0.014
Chrysene	228	436	0.002
Benzo[a]pyrene	252	496	0.0038
Benzo[g,h,i]perylene	276	500	0.00026

The other common characteristics of PAHs are that they are highly hydrophobic and lipophilic, show high octanol-water distribution coefficients and due to their hydrophobicity, they also tend adsorb on to organic particles. These compounds are known to be persistent and also have high accumulation potential. As pure chemicals, PAHs generally exist as colorless, white, or pale yellow-green solids.

1.4.2.2 Sources and Exposure to PAH

The primary sources of exposure to PAHs are inhalation of the compounds in tobacco smoke, wood smoke, and ambient air, and consumption of PAHs in foods. For some people, the primary exposure to PAHs occurs in the workplace. PAHs have been found in coal tar production plants, coking plants, bitumen and asphalt production plants, coal-gasification sites, smoke houses, aluminum production plants, coal tarring facilities, and municipal trash incinerators.

Workers may be exposed to PAHs by inhaling engine exhaust and by using products that contain PAHs in a variety of industries such as mining, oil refining, metalworking, chemical production, transportation, and electrical industry. PAHs have also been found in other facilities where petroleum, petroleum products, or coal are used or where wood, cellulose, corn, or oil are burned. People living near waste sites containing PAHs may be exposed through contact with contaminated air, water, and soil.

1.4.3 Organochlorines Pesticides

Organochlorines are chemicals in which carbon and chlorine are combined. Some organochlorine are found in nature and some are byproducts of combustion and industrial processes, but the vast majority may have been specifically manufactured for a wide range of uses, including pharmaceuticals, pesticides, plastics, and solvents. Most of organochlorines are Persistent Organic Pollutants (POPs).

Aldrin and dieldrin are the common names of two insecticides that are closely related chemically. Aldrin is readily converted to dieldrin in the environment, so these two closely related compounds are considered together by regulatory bodies. Their toxicities do not differ significantly. Aldrin and dieldrin were widely used from the 1950s to the early 1970s, and their primary use was for control of corn pest. Aldrin has

been used as a soil insecticide to control rootworms, beetles, and termites. Dieldrin has been used in agriculture for soil and seed treatment and in public health to control disease vectors such as mosquitoes and tsetse flies. Dieldrin has also had veterinary use as a sheep dip and, along with chlordane, was used in the timber processing industry.

Heptachlor is a broad-spectrum insecticide, the use of which has been banned or restricted in many countries. At present, the major use of heptachlor is for termite control by subsurface injection into soil. Heptachlor is quite persistent in soil, where it is mainly transformed to its epoxide. Heptachlor epoxide is very resistant to further degradation. Heptachlor and heptachlor epoxide bind to soil particles and migrate very slowly. Heptachlor and heptachlor epoxide have been found in drinking-water at levels of nanograms per litre. Diet is considered to represent the major source of exposure to heptachlor, although intake is decreasing. Prolonged exposure to heptachlor has been associated with damage to the liver and central nervous system toxicity.

Dichlorodiphenyhricliloro-ethane (DDT) is an inexpensive, persistent, broad-spectrum, nonsystemic ingested and contact insecticide. From 1946-1972, DDT was the most widely used agricultural insecticide in the world. DDT was commonly used to control insect pests in gardens, orchards, agricultural crops and forests and to control bloodsucking insects and disease vectors. It is still widely used in tropical countries for disease vector control (malaria, yellow fever, trypanosomiasis and typhus) because of its efficiency as a broad-spectrum insecticide and low manufacturing costs. DDT is highly persistent on solid surfaces and readily partitions into animal fats where it has been demonstrated to bioaccumulate and biomagnify. It has been reported in humans at levels which are of potential concern to health. Human exposure can result directly from spraying or through skin contact, inhalation or from ingestion of contaminated foods. DDT and its metabolites display acute and chronic aquatic toxicity, and mammalian chronic toxicity and there is strong evidence of teratogenicity, mutagenicity, and carcinogenicity.

Technical hexachlorocyclohexane (HCH) and Lindane HCH, a pesticide introduced during the 1940s, has two technical grades: technical HCH and lindane. Technical HCH contains only 10 – 12 % of the active ingredient, gamma-HCH, and is predominantly made up of the non-insecticidal isomers alpha-HCH (60-70 %), beta-HCH (5-12 %) and other minor components. Lindane is almost pure gamma-HCH, and is used as an insecticide in agriculture for the control of lice on cattle and grass grub in pasture, and for insect control on vegetables and in orchards. HCH enters the environment through direct application and through non-point sources during manufacturing and disposal. Once in the environment, HCHs persist and have been show to bioaccumulate. HCHs is the most abundant pesticide in the Arctic Ocean. Dietary exposure is the primary route of human exposure where HCH is used on plants and animals, since it is adsorbed from the gastrointestinal tract. The estrogen effects of HCH and its adverse effects upon the male reproductive system have been reported in a variety of animal studies. Because of concentrations of this pesticide in human breast milk, nursing infants are at special risk.

Toxaphene is a complex mixture composed of many congeners with different chemical properties. Toxaphene has been used against insect pests on cotton, tobacco, forests, grains, ornamental plants and livestock. Toxaphene is very persistent in the environment and is transported long distances in the atmosphere. Toxaphene is both acutely and chronically toxic to aquatic species and wildlife. It has also been identified as a potential carcinogen. Human exposure to toxaphene can induce neurological and respiratory dysfunctions, heart dilations, brain haemorrages and death.

1.4.3.1 Sources and Exposure to OCP

Organochlorine pesticides have entered the environment through industrial discharges, the manufacture and use of organochlorine pesticides in agriculture and industry, and disposal of organochlorine pesticides. These organochlorine pesticides are chemically very stable, which means they break down only very slowly and can remain

in the environment for a long time. Organochlorines which are stable and vapour-forming can be carried by air currents for long distances. Eventually they condense and are deposited on land and water, particularly in cold climatic regions. Organochlorine residues have been detected in air, water, soil, sediment, fish, and birds global wide. They have also been found in remote areas, such as open oceans and polar regions. If they contaminate the food supply of animals, the organochlorines become more concentrated as they move up through the food chain. For this reason, the highest levels of organochlorines are found in species at the top of the food chain: human beings, fish-eating birds and marine mammals. They build up in the fatty tissue and stay in the body for a long time because they are only slowly metabolized and excreted. The organochlorine pesticides that have all four properties of persistence, toxic, vapour-forming, and concentrating up the food chain (bio-accumulative), are among the chemicals now referred to internationally as persistent organic pollutants (POPs). These chemicals are a focus of international concern.

1.5 Legislative Measures

While it encourages the usage of sewage sludge, the EU Directive 86/278/EEC regulates its use to prevent harm to the environment, particularly on soil. Table 1.4 shows limit values for concentrations of organic compounds in sludge of different countries.

In Germany, the fertilizer effects of sludges have to be taken into account according to the rules of the German Fertilizer Act and its respective ordinances when sewage sludge is to be used in agriculture activities (Hing *et al*., 1998). It is prohibited to use sludge in fruit and vegetable cultivation, on grassland, in nature conservation areas, in forests and near water catchments wells especially in water protection areas. The US regulation on the use of sewage sludge in agriculture does not establish numerical pollutant limits of any organic pollutants, because at least one of the following criteria

applied for the organics considered, the pollutant is banned for use, has restricted use or is not manufactured for use in the US, the pollutant is detected infrequently in sludge and is present in 5 % of sludge samples, the limit for an organic pollutant derived from the 503 exposure assessment is greater than the 99th percentile concentration in sludge (Hing *et al*., 1998).

Table 1.4 Standard for concentration of organic contaminants in sewage sludge in different countries of the EU (EU, 2000).

	AOX mg/kg dw	DEHP mg/kg dw	LAS mg/kg dw	NP/NPE mg/kg dw	PAH mg/kg dw	PCB mg/kg dw	PCDD/F ng TEq/kg dw
EU 2000	500	100	2600	50	6[1]	0.8[2]	100
Denmark	-	50	1300	10	3[1]	-	-
Sweden	-	-	-	50	3[3]	0.4[4]	-
Lower Austria	500	-	-	-	-	0.2[5]	100
Germany	500	-	-	-	-	0.2[5]	100

[1] Sum of acenapthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo (b+j+k) fluoranthene, benzo (a) pyrene, benzo (ghi) perylene, indeno (1,2,3-c,d) pyrene.
[2] sums of 6 congeners PCB 28, 52, 101, 138,153, 180.
[3] sums of 6 compounds
[4] sums of 7 congeners
[5] each of the six congeners PCB 28, 52, 101, 138, 153, 180.

1.6 Extraction Technique

The sample preparation step in an analytical process typically consists of an extraction procedure that results in the isolation and enrichment of components of interest from a sample matrix (Marsin *et al.,* 2004). Increasing interest in sample preparation research has been generated by the introduction of non-traditional extraction technologies (Wang and Weller, 2006). In this context, the development of more rapid and efficient methodologies for the sample preparation of solid matrices is a significant

trend in modern analytical chemistry (Fifield and Haines, 1997). A classification of extraction techniques that unifies the fundamental principles behind the different extraction approaches is shown in Figure 1.4.

These technologies address the need for reduction of solvent use, fast and environment friendly techniques. These extraction techniques are frequently easier to operate and need further optimization steps. Some of the common extraction procedures for organic contaminants in sewage sludge are as follow;

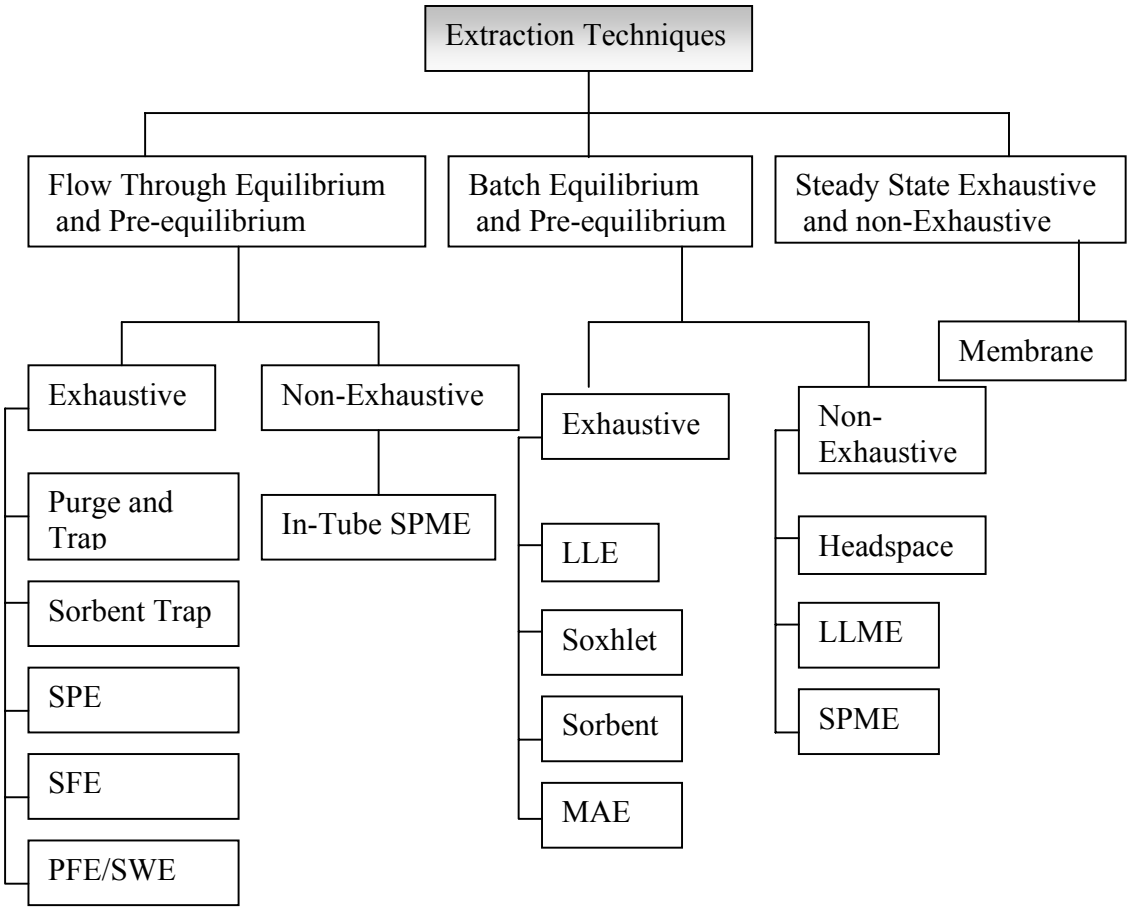


Figure 1.4 Classification of extraction technique

SPE = Solid Phase Extraction, SFE = Supercritical Fluid Extraction, PFE = Pressurized Fluid Extraction, SWE = Subcritical Water Extraction, LLE = Liquid-Liquid Extraction, MAE = Microwave Assisted Extraction, LLME = Liquid-Liquid Microextraction

1.6.1 Basic Theory of Extraction

The fundamental thermodynamic principle of chemical extraction techniques involved the distribution of analyte between the sample matrix and the extraction phase (Pawliszyn, 2001). When liquid is used as the extraction medium, the distribution constant (K_{es}), can be explained by following equation;

$$K_{es} = a_e/a_s = C_e/C_s \quad (1.1)$$

K_{es} defines the equilibrium conditions and ultimate enrichment factors achievable in the technique, where a_e and a_s are the activities of analytes in the extraction phase and matrix, which can be calculated using the appropriate concentrations. For solid extraction phase adsorption, the equilibria can be explained by following equation;

$$K^s_{es} = S_e/C_s \quad (1.2)$$

Where S_e is the solid extraction phase surface concentration of adsorbed analytes and C_s is the concentration of the sample. The above relationship is similar to equation 1.1, with the exception that extraction phase concentration is replaced with the surface concentration. The S_e term in the numerator indicated that the sorbent surface area available for an adsorption must also be considered.

1.6.2 Soxhlet Extraction

Traditional methods such as Soxhlet extraction, which have been used for many decades are very time-consuming and require relatively large quantities of solvents (Castro and Ayuso, 1998). Soxhlet extraction which has been used for a long time is a standard technique and the main reference for evaluating the performance of other solid–

liquid extraction methods. Soxhlet extraction is a general and well-established technique which surpasses in performance of other conventional extraction techniques except for the extraction of thermolabile compounds (Fifield and Haines, 1997).

In a conventional Soxhlet system, the sample is placed in a thimble-holder, and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask the solutes is separated from the solvent through distillation process. The solutes are left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved.

The use of soxhlet extraction to determine quantitatively the level of organic contaminants in the environments is well recognized. The solvents typically used in soxhlet extraction included dichloromethane and a combination of acetone and hexane (Castro 1998). The solvent chosen depends on both the solute and solid. Soxhlet extraction is useful in determining the total pollutant contents in soils, though it is not very efficient. Soxhlet extraction has also been utilised in this laboratory for analysis of PAHs and OCPs, where approximately 10 g of freeze dried sludge sample was subjected to Soxhlet extraction for 16 hours using a mixture of hexane and dichloromethane.

Large volumes of both sample and solvent (300 mL) are necessary for traditional soxhlet extraction. The need to dispose of these solvents presents the potential for further environmental contamination and expense. Soxhlet extraction is also very time consuming. Up to 24 hours may be needed for full extraction and concentration of analytes that are tightly integrated into the soil (Sporring *et al.,* 2005).

Abrha *et al.,* (2000) have conducted a study of PCB recovery from spiked organic matric using soxhlet extraction. Another work reported by Crespo and Yusty (2005) who studied the comparison of SFE and soxhlet extraction for the determination of PCBs in seaweed samples. Hawthorne and co worker, (1998) have studied the

comparison of soxhlet, PLE, SFE and SWE for environmental solids. Sporring *et al.,* (2005) also studied the comprehensive comparison of classic soxhlet, ultrasonic, SFE, MAE and ASE for the determination of PCB in soil. In Malaysia, limited research works on PCB analysis have been carried out. A study on the analyses of PCB in fish tissues have recently been reported (Mohd Sani and Syahidah, 2004).

Some soxhlet extractions are further coupled with saponification techniques to enhance determination of PAHs. Saponification with bases is applied to increase the availability of contaminants in soil by hydrolysis and salvation of organic matter. Codina *et al.,* demonstrated that soxhlet plus saponification techniques has proven to be yield higher recoveries of PAHs as compared to pure traditional Soxhlet techniques. This method was further improved by using silica gel clean-up and fluorescence detection, in preference over UV detection.

1.6.3 Supercritical Fluid Extraction

Supercritical Fluid Extraction (SFE) has attracted researchers interest for the past 20 years, mainly for the extraction of solid samples because it offers short extraction times and minimum use of organic solvents (Chester *et al*., 1992). Supercritical fluid is defined as a substance above its critical pressure, Pc and temperature, Tc resulting in a fluid that is more penetrative and higher solvating power (Castro *et al.,* 1994). CO_2 has been popular because of its low cost, availability, safety and its suitable critical temperature (31.2 °C) and pressure (72.8 atm; 1 atm = 101,325 Pa) (Smith, 1988).

Figure 1.5 shows a typical phase diagram in which for a pure substance the temperature and pressure regions of the substance occurs as a single phase (solid, liquid, gas, supercritical fluid). Such phases are bounded by curves indicating the coexistence of two phases, which determine the point of sublimation, melting and vaporization. The

curves intersect at the triple point (T_p) where solid, liquid and gaseous phase coexist in equilibrium (Smith, 1988).

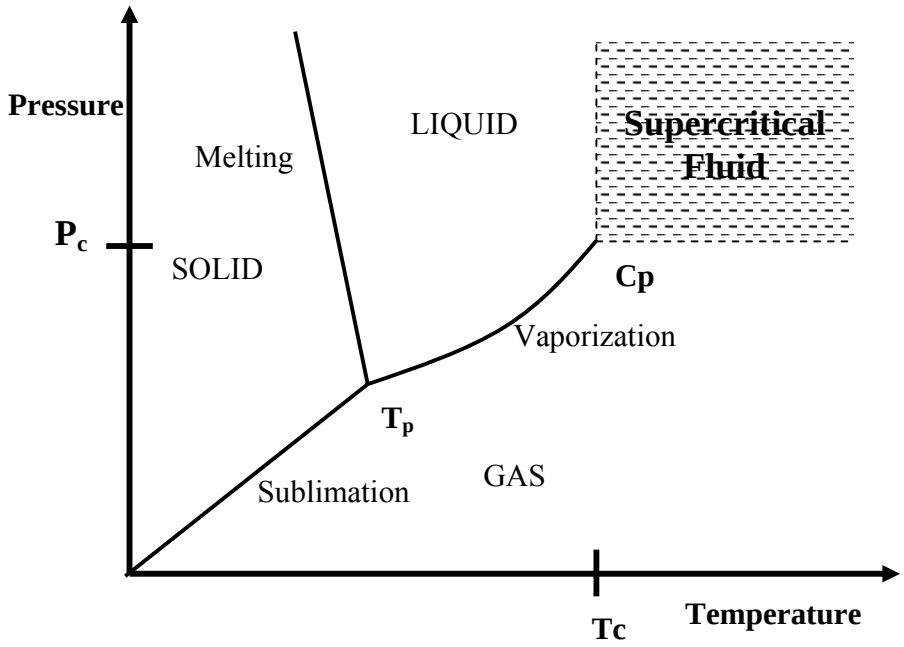


Figure 1.5 A typical phase diagram. Pc is critical pressure, Tc is critical temperature, Tp is the triple point and Cp is the critical point.

The critical point is defined as a point in the phase diagram designated by a critical temperature (T_c) and critical pressure (C_p) above which no liquefaction will take place on raising the pressure and gas phase will be formed upon raising the temperature (Wenclawiak, 1992).The critical pressures of several compounds are given in Table 1.5. Supercritical fluids have physico-physical properties somewhat in between those of liquids and gases (Westwood, 1993). Compared to liquid solvent, supercritical fluids have lower viscosities and higher diffusivities, thus allowing more efficient mass transfer of solutes from the sample matrices.

Table 1.5 Critical points of typical solvents.

Solvent	Critical Temperature Tc (^oC)	Critical Pressure Pc (bar)	Critical Density ρ_c (g/mL)
Carbon Dioxide	31.1	72	0.448
Ammonia	132.4	112.5	0.235
Water	374.15	218.3	0.315
Nitrous Oxide	36.5	71.7	0.45
Xenon	16.6	57.6	0.118
Methane	-82.1	45.8	0.2
Ethane	32.28	48.1	0.203
Ethylene	9.21	49.7	0.218
Propane	96.67	41.9	0.217
Pentane	196.6	33.3	0.232
Methanol	240.5	78.9	0.272
Ethanol	243.0	63.0	0.276
Isopropanol	235.3	47.0	0.273

According to Camel (2001), there are several uses of supercritical fluids. The supercritical fluids can be applied for separation, reactions, analytical domain and more specifically chromatography and extraction purposes such as remediation. The development of SFE is due in part to the patent developed by Zosel in 1970 for decaffeinating green beans with supercritical CO_2 (Taylor, 1996). This process involved soaking the beans in water and submerging them in supercritical CO_2. In the mid 1980s, SFE was believed to be the new solid samples extraction method (Smith, 1999). Several researchers believed this technique to be more selective and cleaner than conventional

organic solvent extraction method (Neude *et al.,* 1998 and Mannila *et al.,* 2002). Rosa and co workers (2005) have reviewed the supercritical technology in Brazil.

In practice, more than 90 % of all analytical SFE is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bar) and temperature (32 °C), CO_2 is non-poisonous, not flammable or explosive, chemically relatively inactive, available in high purity at relatively low cost, easily removed from the extract, and creates no environmental problems when used for analytical purposes. In the supercritical state, CO_2 has a polarity comparable to liquid pentane and therefore, best suited for lipophilic compounds (Smith, 1988).

The second most common choice of extraction fluid for analytical SFE is N_2O. This fluid was considered better suited for polar compounds because of its permanent dipole moment (Hawthorne *et al.,* 1992). One of the applications where N_2O has shown significant improvements when compared to CO_2 is for the extraction of polychlorinated dibenzodioxins from fly ash. Unfortunately, this fluid has been shown to cause violent explosions when it was used for samples contained high organic content. Thus, it is highly recommended to be used appropriately.

SFE has been used for several years for the decaffeination of coffee and tea, in food and seed oil extraction, and in the production of fine powders (Marr and Gamsee, 2000). Recently, the US Environmental Protection Agency (EPA) adopted has SFE as official analytical method for the removal of total petroleum hydrocarbons and PAHs from environmental matrices (EPA Method 3560 and 3561).

Considerable researches on CO_2 extraction of PCBs from the environmental have been aimed towards the optimization of analytical scale extractions for the characterization of PCBs in the environmental samples (Zhu and Lee, 2002). Most of the work was done on soils and sediment samples but very little effort in sewage sludges. Supercritical carbon dioxide extraction proved to be a valuable, fast, quantitative and partly selective extraction technique for determination polycyclic aromatic hydrocarbon

(PAHs), polychlorinated biphenyls (PCBs) and chlorinated pesticides (OCPs) in sewage sludge (Berset and Holzer, 1999). The most recent report on the extraction of organic contaminants from the environmental samples was by using dry ice CO_2 based supercritical fluid extraction (Chiu *et al*., 2005).

An extraction utilizing supercritical fluids can be divided into three sequential steps, which are initial partitioning of the analyte from matrix active sites into the supercritical fluid, elution of analyte from the extraction cell and collection of the analyte in SFE trapping system (Hawthorne *et al.,* 1993). Zhu and Lee claimed that SFE employing CO_2 was easier to perform and was a feasible alternative extraction procedure for monitoring PCBs in pine needle samples. In a similar study, Nilson *et al*., (2002) developed a simple selective SFE method for the determination of PCBs desorption behavior in sediment. In another study by Tong and Imagawa, (1995) they determined the optimum pressure, temperatures, time factors and co solvent composition that enabled maximum extraction of all PCBs.

Van Der Velde *et al.,* (1992) reported the comparison of SFE with two other techniques widely use for the extraction of PCBs and OCP in soil. SFE using CO_2 at 50 ^{o}C and 20 Mpa 10 min static followed by 20 min dynamic extraction with collection in *iso*-octane, was found to give the optimum extraction of PCBs from soils. Chen *et al.,* (1997) studied about the remediation of PCBs contaminated soils and sediments by SFE using condition of 30 min, 40 ^{o}C and 100 atm. Reutergardh *et al.,* (1998) used SF CO_2 to extract three planar (PCB 77, 169 and 126) and three mono-ortho (PCB 105,156 and 189) PCBs from three different soil types. Bowadt *et al*., (1997) have developed a single SFE method for field extraction of PCBs in soils for use in combination with capillary GC-ECD without the need of clean up steps after extraction.

PCBs extraction from biological matrices (milk, fat, tissues, and food) is another important area of SFE. Ramos *et al*., (2000) studied the distribution of PCBs in milk fat globules by sequential extraction of four different lipidic fractions from powdered full fat milk with supercritical carbon dioxide. In another research by Atuma *et al.,* (1998),

they have determined non-ortho PCBs 77, 126 and 169 in a number of fish species from Swedish coastal environment, using either supercritical fluid extraction or the traditional liquid-liquid extraction followed by HPLC separation aqueous solution. Umi, (1994) has studied the application of SFE to the analysis of selected zingiberaceae species.

Cleanliness and safety are of the main advantages of SFE since it is non-toxic, poses no fire risk and does not introduce more hazardous waste into the matrix. SFE is also typically faster than conventional liquid extraction techniques since supercritical CO_2 is capable of penetrating the soil much faster than liquids (Chester, *et al.,* 1996; Taylor, 1996; Wenclawiak, 1992). Finally, SFE provides selectivity and it can remove a wide range of contaminants such as PCBs, PAHs and heavy metals without extracting integral components of the soil such as humic acid (Castro and Carmona, 2000). SFE also has several disadvantages that impede its application in the field, especially the cost of instrument.

1.6.4 Pressurized Liquid Extraction

Pressurized liquid extraction (PLE) is also known as pressurized fluid extraction (PFE), pressurized solvent extraction (PSE), accelerated solvent extraction (ASE) or enhanced solvent extraction (Ramos *et al.,* 2002). Pressurized liquid extraction (PLE) has been gaining popularity as an extraction technique compared to conventional techniques such as liquid–liquid extraction, soxhlet extraction, ultrasonic extraction etc., which use large amounts of solvent, and are tedious, time-consuming and labour intensive (Ong *et al.,* 2003). PLE has demonstrated advantages for automation, reduced extraction time, and lower solvent use than for conventional Soxhlet extraction (Burkhardt *et al.,* 2005).

Recently, PLE with subcritical heated water (PLEHW) has been used for extracting polar to moderately polar organic compounds from sediment samples. At

temperatures greater than 250 ^{o}C, extraction of nonpolar high molecular weight compounds, such as PAHs, PCBs, and brominated flame retardants, has been reported. The solubility of solutes in subcritical water increases dramatically with increasing temperature and results from the decreasing dielectric constant (ε) of water (Burkhardt *et al.,* 2005).

Several papers have been successfully reported for the application of PLE, such as for PAHs, OCPs, in environmental samples. However, few papers have been published using PLE for determining PAHs in sewage sludge. The use of higher extraction temperature and the role of high pressure for PLE reported to give comparable or higher extraction efficiencies compared with other extraction techniques (Helaleh *et al.,* 2005). Higher temperature >140 ^{o}C was not recommended, which leads to the co-extraction of other organic substances or formation of degradation products of the parent compounds. However, the use of high pressure keeps the solvent in the liquid phase and at high temperature facilitates a better penetration of the solvent into the sludge matrix. Therefore, PLE provide higher extraction efficiencies compared with other techniques (Helaleh *et al.,* 2005).

In a study conducted by Schantz *et al*., (1997), PLE has been evaluated and found suitable as an alternative to Soxhlet extraction for use in the extraction of PAHs, PCBs and OCP pesticides from natural matrix environmental conditions. It was also demonstrated that soxhlet extraction with methylene chloride is not quantitative for higher molecular weight; therefore PLE is the preferred technique for the certification of these materials.

1.6.5 Solid Phase Extraction

In recent years, solid-phase extraction (SPE) has become an increasingly popular procedure in environmental analysis. SPE is a popular procedure that is used to pre-

concentrate components to be analyzed and clean-up matrices from sample for analysis (Sun *et al.,* 1998)

SPE is a sample treatment technique that passes a liquid sample through a sorbent. The above technique can fulfill two functions. In the first case, the analytes are eluted in a small volume of a solvent and thus, the analytes are concentrated. In the second case, the function of the solid-phase extraction is to clean the sample. One of the benefits of using this method is that only small volumes of the solvent are required and the purification time is short. It also allows for a wide variety of extraction conditions that may be used to achieve the desired separation and pre-concentration (Oleszczuk and Baran, 2004).

In contaminated soil, besides the PAHs, PCBs and OCP that are analyzed, other compounds may interfere with the determination. In addition, some PAHs, PCBs and OCP in the extraction solution from the soil cannot be determined directly because of their lower concentrations. Therefore, SPE as a clean-up procedure is often required for the determination of PAHs in soil samples, while aqueous samples need pre-concentration (Sun *et al.,* 1998).

1.6.6 Solid Phase Microextraction

Pawliszyn and coworkers devised solid-phase microextraction (SPME) as a new extraction technique in 1989. This technique uses a thin polymer film coating to extract analytes from aqueous or gaseous samples. Then, the fiber is inserted directly into the injector of a gas chromatography system, and the extracted analytes are thermally desorbed and analyzed. SPME can integrate sampling, extraction, preconcentration and sample introduction into a single step (Mohammadi *et al.,* 2005).

Two strategies of SPME, direct-immersion and headspace, have been applied to the analysis of a wide variety of pollutants. Direct-immersion has been used to analyze volatile organic compounds, pesticides, PCBs and PAHs in water and soil samples. Headspace has also been used to determine organophosphorus pesticides in soil, and PAHs in water and soil samples (Pino *et al.,* 2003).

In the SPME have a number of advantages over other water extraction techniques such as solid-phase extraction or liquid–liquid extraction. It uses little or no organic solvent, which is clearly of environmental benefit. Many of the more traditional extraction techniques involve multi-step procedures that always present the risk of analyte loss, while SPME achieves contaminant extraction and concentration in a single step thus reducing this risk. The technique is also relatively inexpensive with a single fiber being capable of performing between 50 and 100 extractions (King *et al.,* 2004)

Nowadays, SPME is among the most recommended techniques, employed for the extraction and pre-concentration of volatile and semi-volatile compounds at trace levels in a variety of matrices (Mohammadi *et al.,* 2005).

1.6.7 Subcritical Water Extraction

Like CO_2, water is an "environmental-friendly solvent" and has the additional advantages of being readily available, non-toxic, and cheap. Water is a unique solvent because of its highly hydrogen bonded structure, and at room temperature it has a disproportionately high boiling point for its mass, a high dielectric constant and high polarity. Subcritical water extraction (SWE), also known as hot water extraction, pressurized (Hot) water extraction, high temperature water extraction, superheated water extraction or hot liquid water extraction, is an emerging technique based on the use of water as an extraction solvent at temperatures between 100 and 374 ^{o}C (critical point of

water, 374 ^{o}C and 22 MPa) and at a pressure which is high enough to maintain in the liquid state (Yang *et al.,* 1998).

Water is a unique solvent because of its higher hydrogen bonded structure. In addition, ambient temperature it has a disproportionately high boiling point for its mass, a high dielectric constant and polarity. However, when water is heated, its properties change markedly as the hydrogen bonded lattice is disrupted due to an increasing in thermal motion (Meyer, 1993). Pure water at ambient temperature and pressure has electric constant, ε = 79 however, when the temperature is increased to 250 °C at a pressure of 5 MPa (necessary to maintain the liquid state) a significant reduction of electric constant value. (ε = 27) (Meyer, 1993). This value is similar to that of ethanol at 25 °C and 0.1 MPa and consequently, low enough to dissolve many compounds of intermediate or low polarity (Yang *et al.,* 1997).

The polarity of water can be controlled over a similar range by simply heating pure water with enough pressure to maintain the liquid state (Figure 1.6). While water at ambient conditions has very high surface tension and fairly high viscosity, a high temperature will reduces surface tension and viscosity (Ramos *et al.,* 2002). Heating water at 250 °C reduces its surface tension about the same as solvent programming to 100 % methanol or acetonitrile (as in liquid chromatography) and reduces the viscosity of water even more than solvent programming to 100 % methanol or acetonitrile. Thus, the mobile phase polarity, surface tension and viscosity can be controlled by simply heating pure water (in the liquid state) over the same ranges achieved by conventional solvent programming to 100 % methanol or acetonitrile (Smith,, 1988).

When water is heated at low temperature, inert or dipole bonding between analytes and matrices is broken whereas a high temperature is required to break Van Der Waals forces and even higher temperatures are needed to break π-π electronic interactions. Yang *et al*., (1998) examined the solubility of a number of PAHs and found marked changes in solubility as the temperature increased. The high degradation efficiency achieved using metal additives in water at reasonable temperatures and

pressure demonstrates the potential for subcritical water dechlorination of PCBs in paint scrapings and potentially in other solid and liquid wastes (Kubatova *et al*., 2003). Richter *et al.,* (2003) have studied the determination of pesticides in soil using continuous subcritical water extraction and all pesticides can be screened depending on their polarity.

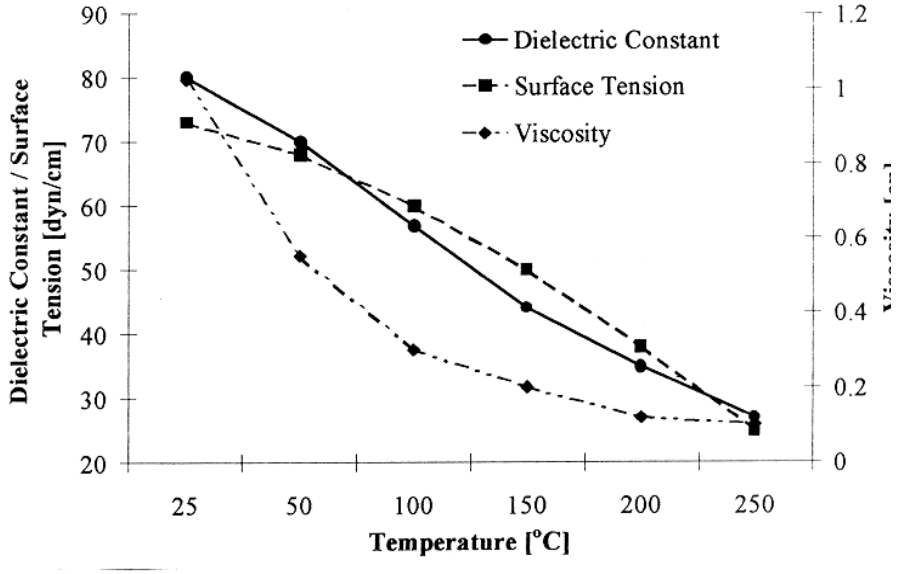


Figure 1.6 Temperature influence on physical properties of water

Ruziyati and Smith, (2005) have studied an on-line coupled superheated water extraction and superheated water chromatography. Hartonens *et al.,* (1997) reported good recovery of PCBs was achieved with the tenax trap, solid phase (florisil) and liquid collection PCBs were recovered with higher efficiency than soxhlet extraction. Another work by Kubatova *et al*., (2001) showed the comparison of subcritical water and organic solvents for extracting kava lactones from kava root. About 40 minutes of subcritical water extraction yielded essentially the same recoveries of lactones employing 18 hour of sonication.

SWE can efficiently elute both polar and non-polar solutes from normal and reversed phase packing based on the lower polarity and surface. Hawthorne *et al.,* (1998) reported on a simple method for estimating PCBs concentrated on soils and sediments using subcritical water extraction coupled with solid phase membrane extraction. Konda *et al.,* (2002) employed subcritical water extraction to evaluate the desorption behavior of organic pesticides in soil.

Lamm and Yang, (2003) reported the off line coupling of subcritical water extraction with subcritical water chromatography via a sorbent trap and thermal desorption. They demonstrated that the off-line coupling technique was efficient and successful. The extract of anilines and phenols in sand has yielded an approximately 80 % recovery. Hinz *et al*., (2000) studied on the remediation of a nonachloro biphenyl congener with zero-valent iron in subcritical water. Pross *et al.,* (2000) conducted an extraction of PCBs with supercritical fluid carbon dioxide, sulfur hexafluoride and subcritical water. CO_2 and water were found to be suitable for the quantitative extraction of all PCBs. However, water was found to be the best solvent because it is cheap, good availability and environmental safety.

In most of the extraction studies, distilled or deionised water has usually been used alone as the solvent in either static or dynamic extraction modes. Sometimes it is degassed or flushed with nitrogen to remove oxygen to prevent oxidative side reactions. pH control has been employed to enhance the extraction of selected analytes. For example, Crescenzi *et al.,* (2000) found improved extraction efficiency for a range of polar and medium polar pesticides, if a phosphate buffer at pH 7.5 was employed as the superheated eluent and this condition could release the sequestered pesticides from aged soils. In other studies, Pérez and Castro, (2000) added dodecyl sulfate (SDS) as a micellar agent to the extraction water to enhance the extractability of PAHs and thereby reduce the extraction temperature and time required.

The basic components for static SWE are normally used for continuous SWE. After filling the extraction cell with the sample, the cell is placed in a heated oven. An

experiment typically starts with pumping the water at selected flow rate until the selected pressure for SWE is reached. The extracted analytes are collected in a solvent or using other collection technique.

1.7 Extract Clean-Up

The determination of PCBs, OCP, PAHs in soil, sediment and sludge samples require a good clean-up, while aqueous samples need concentration due to their low concentration levels. This is due to PCBs, OCP and PCBs, which are present in sludge in the range of ng/g or below (Folch *et al.,* 1996; Preira and Kuch, 2005). Two most commonly used techniques for sample clean up are the conventional column chromatography and solid phase extraction (SPE) utilising disposable cartridges.

Silica gel is often used in column chromatography for the separation of analytes from interfering compounds of a different chemical polarity (Jang and Li, 2001). It is used deactivated, after heating to 130 to 160 ^{o}C. Generally conventional column chromatography need larger amount of adsorbent and larger volume elution solvent, but with a greater clean-up capability (Martinez *et al.,* 2005). In the standard column clean-up protocol, the column is packed with the required amount of adsorbent, topped with a water adsorbent and then loaded with the sample to be analyzed. Elution of the analytes is accomplished with suitable solvent that leaves the interfering compounds on the column and the eluate concentrated off using nitrogen gas.

In a study employing silica gel for the clean up of PCBs from sewage sludge, it was found that the use of silica gel to clean up extract was highly suitable for PCB determination (Martinez *et al.,* 2005; Jang and Li, 2001). The silica gel column chromatography method was also used for the clean-up of sludge samples after extraction by Soxhlet. The same study compared the efficiency of the column to that of SPE cartridges. It was found that the silica column yielded better results in terms of

lesser interfering peaks but this technique needed larger volume of solvent as compared to SPE cartridge (Folch *et al.,* 1996).

SPE cartridge is a popular technique for pre concentrate and clean up matrices from samples. The cartridge column protocol uses SPE cartridges packed with 1 g or 2 g of adsorbent. The SPE cartridge is placed onto vacuum manifold and the cartridge is solvent washed immediately prior to use. After that, the sample extracts are loaded onto the cartridges, which are then eluted with suitable solvent. The collected eluate may need further preconcentration prior to analysis.

1.8 Instrumental Analysis

Various instrumental analytical procedures have been employed for the determination of PCBs, PAHs and OCP pesticides. Gas chromatography (Alvarado *et al.,* 1997), high performance liquid chromatography and capillary electrophoresis are the techniques most commonly used.

1.8.1 Gas Chromatography

Gas Chromatography (GC) is a technique that can completely separate the whole range of organic contaminant as well as detecting minor compounds with potential high separation efficiency and good sensitivity. The most widely used gas chromatographic detector for PCBs and OCP pesticides is the electron capture detector (ECD) (Bowardt and Johansson, 1994, Booij *et al*., 1998, Rissato *et al.,* 2005). ECD provides an excellent linear response, sensitivity and reliability. However, the separation of all PCBs and OCP remains quite a challenge, even with high resolution capillary GC. The most widely use gas chromatographic detector for PAHs is flame ionization detector (FID). This is a

result of its universally accepted characteristics of excellent response linearity, sensitivity and reliability. There are various studies that have employed the use of GC-FID (Moreda *et al.,* 1998; Bodzek *et al.,* 1997; Hawthorne *et al.,* 1992). This method of dectection is mostly used for determination of PAHs.

1.8.2 Capillary Electrophoresis

Capillary electrophoresis (CE) methods including capillary zone electrophoresis (CZE) have recently emerged as the most efficient methods available for the separation of PCBs components in a mixture. Electrokinetic chromatography with cyclodextrin derivatives (CD-EKC) was used to achieve the rapid enantiomeric separation of PCBs by electrokinetic chromatography using mixtures of neutral and charged cyclodextrin derivatives (Garcıa *et al*., 2001). In the study reported by Garcia *et al.,* (2001), thirteen out of the nineteen chiral PCBs which were stable at room temperature were individually separated into their two enantiomers by using 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.5) containing carboxymethylated γ-cyclodextrin (CM-γ-CD) as pseudostationary phase.

However, CZE is limited to the analysis of water-solube, charged species and is therefore not applicable for analysis of neutral and water-insoluble PAHs (Brown et al., 1996). Therefore, for the separation of PAHs, which are all neutral, non-ionizable and are of similar hydrophobicity, it is necessary to extend the concept of employing a mobile phase and pseudostationary phase to the utilization of buffer modifier (Yik *et al.,* 1992). One of the most successful schemes for improving the partitioning for PAH separation is the addition of cyclodextrins to the buffer.

1.8.3 High Performance Liquid Chromatography

PAHs are commonly analyzed by HPLC with fluorescence detection. Although capillary gas chromatography is known to have much higher resolution than HPLC methods, but nowadays, HPLC can easily separate the 16 EPA priority PAHs (Harrak et al., 1996). This method of analysis is known to be selective and requires less clean-up procedures as compared to GC-MS due to the use of the guard precolumn in the HPLC. A number of studies have been conducted to determine PAHs using HPLC (Codina et al., 1994; Miege et al., 1999; Popp et al., 1997; Perez et al., 200).

Krup *et al.,* (1977) studied the analysis of PCBs by high-performance liquid chromatography (HPLC) and capillary gas-liquid chromatography. A method to separate PCBs by using HPLC was developed by Lundgren *et al.,* (2002). The HPLC column was packed with Amoco PX-21 activated carbon dispersed on octadecylsilane (ODS). The separation was carried out by gradient elution with *n*-hexane, dichloromethane and toluene in the forward direction followed by reversed elution with toluene. The developed immunosensor assay was able to detect PCB congeners in the range of concentrations usually found in the real matrices (Přiby *et al.,* 2006).

1.9 Problem Statement

Municipal sewage sludge and wastes have become a major problem in many countries in the world including Malaysia due to rapid population growth and urbanization. Approximately 3 million cubic meters of sewage sludge is produced annually in Malaysia and the total cost of managing it was estimated at RM one billion (Kadir and Haniffa, 1998). By the year 2020, the volume is estimated to increase to 7 million cubic meters, which will require about double the KLCC twin tower to be filled or almost 1.4 million tanker trips to manage (Velayutham and Kadir, 1999). An

agricultural use of quality sludge is widely practiced in the United Kingdom and the European Union, but is rarely practiced in Malaysia.

The presence of measurable quantities of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn), toxic organic compounds and pathogenic organisms in sewage sludge render its disposal a major problem (EU 2000). Almost all sludge based research in Malaysia has concentrated on the inorganic aspects of contamination, particularly heavy metals. However, little attention is given to the determination of organic pollutants in sewage sludge, although these organic compounds, namely PCBs, PAHs and OCP are toxic and hazardous.

Traditionally, Soxhlet extraction technique has been employed for PCBs, PAHs and OCPs determination. This technique is often time-consuming and requires large volumes of organic solvent. The use of large volumes of extracting solvents adds to the additional cost associated with purchasing and disposal of toxic solvents. For these reasons, alternative extraction strategies are much needed especially those involving supercritical fluid CO_2 and subcritical water extraction. Certain countries have introduced standards of tolerance for PCBs, PAHs and OCPs values allowable in sludge before it can be used for soil improvement. However there is no regulation in the Malaysian legislative system defining the permissible levels of organic compounds in the environment.

The objective of a specific guideline for sludge is to ensure the application of municipal sewage sludge onto agricultural lands is done in a beneficial and environmentally acceptable manner, as well as protecting the environment and human health from adverse effects. This study is therefore undertaken to address the above said problems by developing suitable extraction and instrumental techniques for the analysis of selected PCBs, PAHs and OCPs in sludge samples.

1.10 Objectives of Research

The objectives for this research are as follows;

(i) To develop extraction strategies of organic pollutant from sludge samples.
(ii) To characterize sewage sludge from other aspects such as pH, moisture contents, total vapor and solid, nutrient content (Nitrogen, Phosphorus, Potassium) and heavy metals.
(iii) To determine organic pollutant levels in real municipal sewage sludge.

1.11 Scope of Research

The scope for this research encompasses the analysis of three types of organic pollutants commonly found in sewage sludge. Real sludge samples are those obtained from Indah Water Konsortium (IWK) treatment plants in Johore area.

CHAPTER 2

EXPERIMENTAL

2.1 Introduction

This chapter describes in detail the chemicals, apparatus and instrumentation used in the study. General experimental procedures are also presented in this chapter.

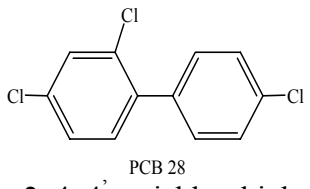
2.2 Chemicals and Material

The chemicals, apparatus and instrumentations that were used in the extractions and analytical procedures, are listed in Section 2.2.1 and 2.2.2 as follows;

2.2.1 Chemicals

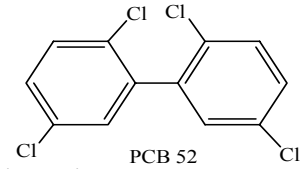
PCB standards (IUPAC No PCB 28, 35, 52, 101, 118, 153, 138 and 180) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) at a certified concentration of 10 μg/mL in iso-octane. Methanol was obtained from Merck (Darmstadt, Germany), iso-

octane from BDH Limited (Poole, England), whereas *n*-hexane was obtained from Fisher Scientific (USA).



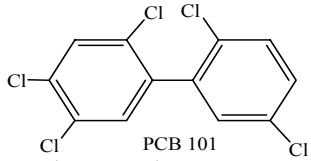
PCB 28

2, 4, 4' - trichlorobiphenyl



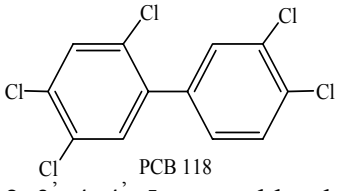
PCB 52

2,2', 5, 5' - tetrachlorobiphenyl



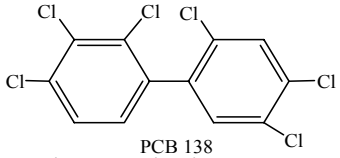
PCB 101

2, 2', 4, 5, 5' -pentachlorobiphenyl



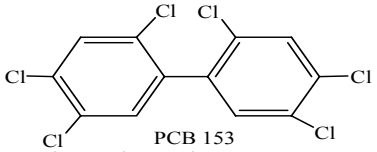
PCB 118

2, 3', 4, 4', 5- pentachlorobiphenyl



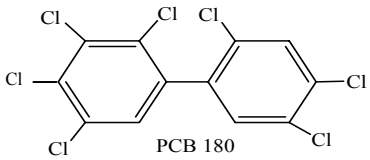
PCB 138

2, 2', 3, 4, 4', 5' - hexachlorobiphenyl



PCB 153

2, 2', 4, 4', 5, 5' - hexachlorobiphenyl



PCB 180

2, 2', 3, 4, 4', 5, 5' - heptachlorobiphenyl

Figure 2.1 Molecular structures of seven PCBs used in the present study.

Two individual PAH standards of naphthalene and flouranthene of analytical grade were obtained from Riedel-de Haen, Germany. Benzo(a)pyrene and

benzo(b)fluorene were obtained from Fluka, Switzerland, and phenanthrene was obtained from BHD Chemical, Germany. OCP standards of dieldrin and TDE (1,1-dichloro-,2-bis (4-chlorophenil)etane) were obtained from Polyscience, Illinois, USA. Lindane, chloropyrifos, pp-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)etane) and heptachlor were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All other metal salts and reagents were of analytical grade. Liquid CO_2, nitrogen and helium was industrial grade from Malaysian Oxygen Berhad (MOX), Pasir Gudang, Johore, and solid phase extraction disposable cartridges LC-18 (6 mL) from Supelco (Bellefonte, USA). Nitric acid, hydrochloric acid, boric acid, potassium sulfate, hydrogen peroxide, silica gel 60 and *n*-octanol were obtained from Merck, Germany. Anhydrous sodium sulfate, sodium persulfate, sodium hydroxide and bromocresol/methyl red were obtained from BDH, England. Copper powder was obtained from Ajax Chemical, Australia and Phos Ver 3 reagent was obtained from Hach Company, USA. Double-distilled deionized water of at least 18 MΩ was obtained using a Millipore Water System (France).

2.2.2 Apparatus and Instrumentations

The Soxhlet apparatus set was obtained from Quickfit, England. The resultant extracts were evaporated using a rotary evaporator from Heidolph (Germany), and further concentrated off using nitrogen gas generated using a nitrogen generator from Peak Scientific. A spectrophotometer model Hach DR 4000U (USA) was used for determination of phosphorus content in sludge. The pH meter used in this study was obtained from CyberScan pH/ion 510 Butech Instrument. An atomic absorption spectrophotometer (Perkin Elmer Analyst 400) was used for determination of Copper (Cu), Zinc (Zn), Lead (Pb), Cadmium (Cd) and Nickel (Ni).

The SFE apparatus consisted of a Jasco PU-1580 intelligent HPLC pump (Jasco Cooperation, Tokyo, Japan) fitted with a cooling jacket to deliver CO_2 (Figure 2.2). In order to give a cooling effect to the pump head, ethylene glycol and water mixture

(50:50, v/v) was circulated through the cooling jacket using a chiller and circulator, which delivered the coolant down to -6 ^{o}C. A 5 mL extraction cell was used as the extraction vessel. A Jasco CO-1560 Intelligent Column Oven (Jasco Cooperation, Tokyo Japan) was used to maintain a constant extraction temperature (temperature limit 80 ^{o}C) while a dynamic mixer (Jasco HG1580-32) was utilised for mixing organic modifier and liquid CO_2. A Jasco back pressure regulator (BP-1580-81) was used to control the extraction pressure.

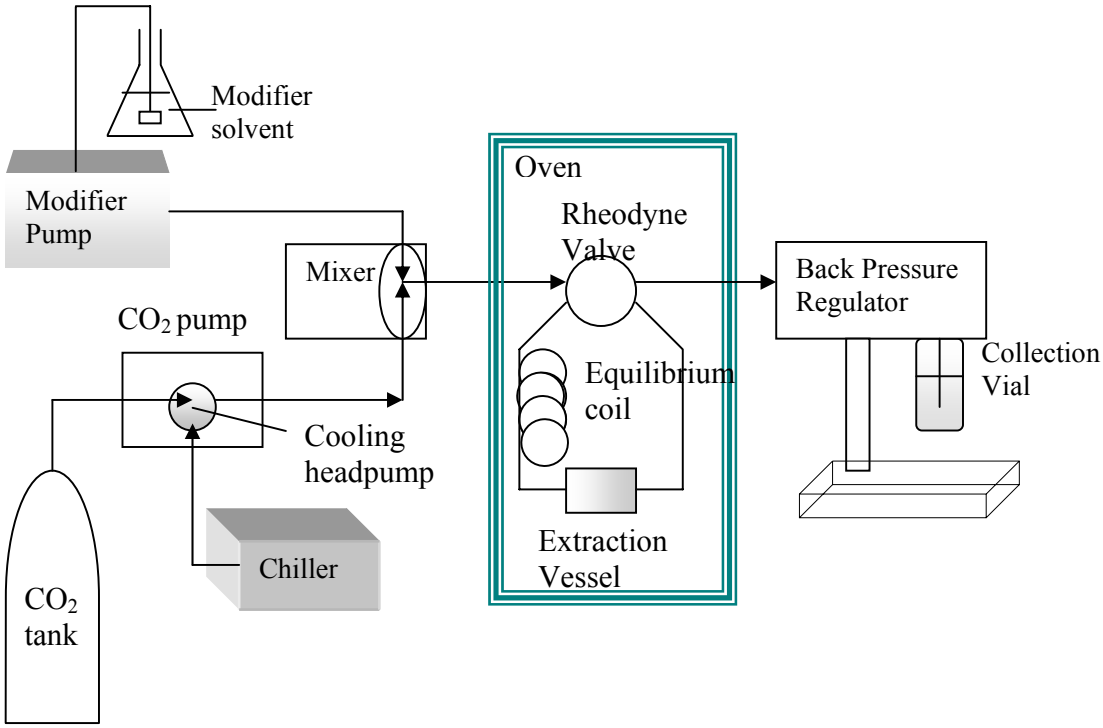
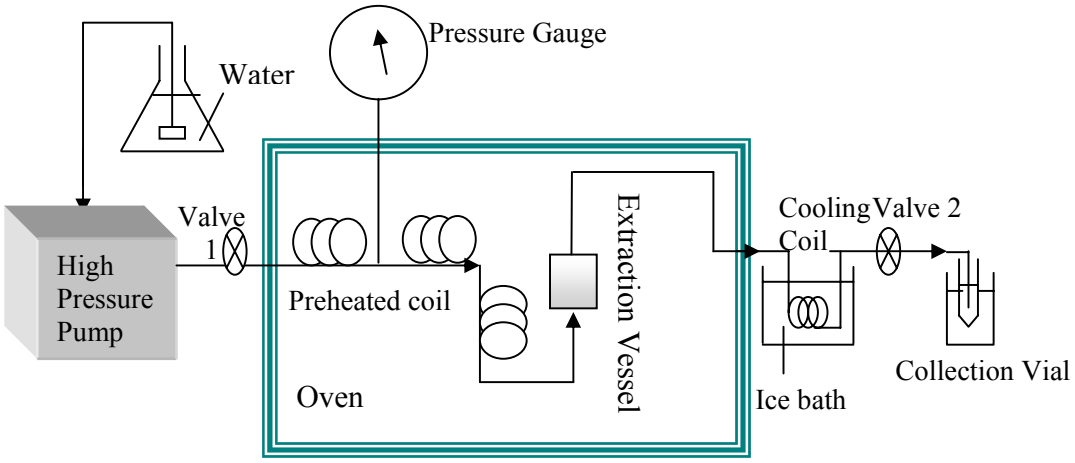


Figure 2.2 Schematic diagram of the basic set up for SFE

The schematic set-up of the lab made SWE unit is shown in Figure 2.3. All connecting tubing (1/16 inch) were made from stainless steel. Connections were made using Swagelok fittings. The system consisted of a HPLC pump (Waters 515, USA) to deliver the extractant (water), and a Shimadzu gas chromatography oven (Tokyo, Japan)

to raise the extraction temperature to the subcritical state (oven temperature limit of 300 ^{o}C). A preheated coil (3 m stainless steel tube, 1/16 inch) was placed in the oven and was followed by a 1.0 mL extraction vessel. A pressure gauge (0 – 6000 psi) from Omega Engineering, Inc. was needed to measure the extraction pressure of the system. A home-made tapered glass flask was used to collect the extract. Two shut-off valves from Whitey Swagelok were used.

(A)



(B)

Figure 2.3 Diagram showing (A) Schematic diagram and (B) actual set-up of the lab-made SWE system.

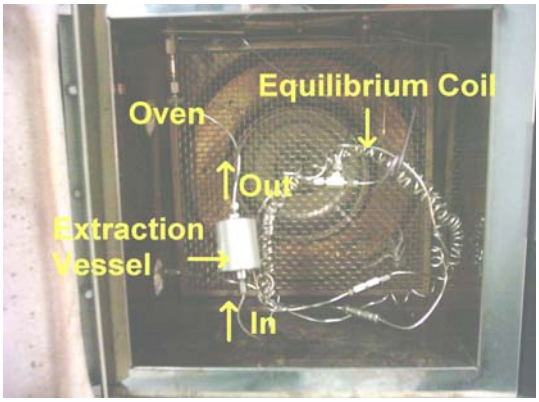


Figure 2.4 View of the inside of the extractor oven

PCBs analyses were carried out using a Perkin Elmer XL GC equipped with an electron capture detector (ECD) and data processing using Chromatography Version 4.1 software. Nitrogen was used as the make up gas for ECD at a flow rate of 30 mL/min and helium as the carrier gas at a pressure of 14.0 psi resulting in a flow rate of 1.0 mL/min. An HP-5MS column (30 m x 0.25 mm ID x 0.25 µm film thickness from Hewlett Packard, USA) was used. Injection port temperature was set at 280 ^{o}C and detector temperature at 290 ^{o}C. The temperature was initially held at 200 ^{o}C for 3 minutes, and then programmed from 200 ^{o}C to 230 ^{o}C at a ramp of 5 ^{o}C/min. The temperature was again held for 3 minutes at 230 ^{o}C and then increased to a further 260 ^{o}C at 2 ^{o}C/min.

GC-MS-SIM (Agilent Technologies 6890N) with Agilent Technologies 5973 MS detector was used for a further identification of all PCB congeners as well. Separation was achieved on an Ultra-1 column (30 m x 0.25 mm ID x 0.25 µm film thickness from Agilent Technologies). The analysis was conducted according to the following temperature program: 40 ^{o}C/min to 40 ^{o}C, 5 ^{o}C/min to 120 ^{o}C, during 15 min to 280 ^{o}C (Sulkowski and Rosinska, 1999). The following ions were used for extracted ion profiling; PCB 28 m/z 258, 256, 150; PCB 52 m/z 282, 290,257; PCB 101 m/z 326,

324, 254; PCB 118 m/z 326, 324, 254; PCB 138 m/z 360, 358, 292; PCB 153 m/z 360, 358, 292 and PCB 180 m/z 394, 392, 326.

2.3 Sewage Sludge Samples

Real sewage sludge samples were collected from seven different IWK municipal sewage sludge treatment plants in Johor.

2.3.1 Sampling and Sample Pre-Treatment

The sampling points chosen in this study were Indah Water Konsortium Johor municipal wastewater treatment facility in Taman Desa Kluang (TD), Taman Intan Kluang (TI), Lima Kedai (LK), Seri Alam Masai (SA), Kawasan Perindustrian Senai 3 & 4 (KIS), Taman Yayasan Segamat (TY) and Taman Seruling Segamat (TS). The map of sampling sites in Johor is presented in Figure 2.5.

The criteria of sewage sludge sampling point were based on the location of industrial activities near the wastewater treatment plant (WTP). Three major areas of Johor were considered namely in Johor Bahru, Kluang and Segamat. In Johor Bahru district, three WTP chosen were located at Taman Perindustrian Ringan Datuk Yunus Sulaiman, at Lima Kedai, Skudai (LK), Seri Alam at Masai (SA) and Kawasan Perindustrian Senai 3 & 4 (KIS). Lima Kedai is a tertiary industrial park situated in Skudai, Johor. Several factories located in that area included that of electronic, timber, hardware, food industries and also from domestic or household activities. Seri Alam at Masai area is also an upcoming medium industrial park. There are a few factories in this area such as printing, metal, concrete, plastic, welding, oil palm process and electronic industries. The third sampling point in Johor was Kawasan Perindustrian Senai 3 & 4.

This area constitutes the medium industrial activities such as battery, electronic, metal and plastic manufacturing.

Figure 2.5 Map of sampling sites for sewage sludge.

In Kluang, the sewage sludge samples were collected at two WTP situated at Taman Intan and Taman Desa. WTP at Taman Desa received wastewater from medium industrial activities such as plastic, mechanic, metal, paper, electronic, paint

manufacturing and also domestic effluent from nearby resident area. However for Taman Intan, the WTP managed wastewater from domestic sources only.

At Segamat area, sludge samples were collected at WTP in Taman Yayasan and Taman Seruling. Taman Yayasan is a medium industrial area where the WTP manages the wastewater from electronic and plastics industries, and domestic sources. However for Taman Seruling, the WTP received wastewater from domestic sources only. The detail map of each sampling point are shown in Appendix A. Table 2.1 summarizes the sampling location, and population equivalent of each WTP. These abbreviations will be used throughout the thesis.

Table 2.1 Sampling locations, sources of wastewater and PE of WTP

Sampling Locations	Sample code	Sources of wastewater	PE
1. Seri Alam Masai	SA	D, I	4264
2. Kawasan Perindustrian Senai 3 & 4	KIS	D, I	2000
3. Taman Desa Kluang	TD	D, I	3200
4. Taman Intan Kluang	TI	D	4000
5. Taman Yayasan Segamat	TY	D, I	4052
6. Taman Seruling Segamat	TS	D	580
7. Perindustrian Ringan Datuk Yunus Sulaiman Lima Kedai	LK	D, I	2000

D= Domestic, I= Industries, PE= Population Equivalent, NA= Not available

All sludge samples were taken from the drying bed of the WTP except for samples TD, TI and TY. The sludge sample from the drying bed was taken using a garden spade. The slurry sludge samples were collected using a long plastic pole called the sludge judge, which was done by the IWK personnel. The sludge samples were collected randomly from both the oxidation pond and the drying bed. All the samples

were air-dried at room temperature for a week. The samples were then ground and sieved through a 2 mm sieve and stored in sample bottles.

2.4 Characterization of Sewage Sludge

In order to characterize the samples, conventional analytical techniques as well as regulatory and modified leaching tests were applied, which included the following:

Sewage sludge were characterized by pH, moisture content, percentage of solid and volatile solids (Mc Keague, 1976). Chemical assays of the sludge samples were determined by acid digestion (Aqua Regia solution; HCI/HNO_3 3:1) and subsequent measurement of the elements in the solution using atomic absorption spectrophotometer (AAS). For the measurement of phosphorus in sludge, UV Spectrophotometry (Hach DR 4000) was applied. All determinations were conducted in triplicate. Total nitrogen was determined by digestion and subsequent measurement by means of the Kjeldahl method (Bremer and Mulvaney, 1982).

2.4.1 Determination of Sludge pH

pH of air-dried sludge was measured using a 1:2.5 ratio of sludge : distilled water mixture. 10.00 g of sludge was placed in a beaker and 25 mL of distilled water was added into it. The mixture was left overnight at room temperature and the pH of the solution was measured using a digital pH meter. Prior to experiments, the pH meter was calibrated using pH 4.01, 7.00 and 10.01 standard buffer solutions (USA standard buffer).

2.4.2 Percentage of Moisture Determination

An empty crucible was weighed before it was filled with 10 g of sludge (W_1) and reweighed. Consequently the crucible containing the sample was heated in a furnace at 105 ^{o}C for 24 hours. After the sample has cooled down, it was weighed once again (W_2). Percentage of moisture in the sample was calculated using the following equation (Equation 2.1);

$$\% \text{ of Moisture} = \frac{W_1 - W_2}{W_1} \times 100\ \% \qquad (2.1)$$

2.4.3 Determination of Total Solids and Volatile Solids

In this experiment, the crucible was first heated at 105 ^{o}C for 1 hour prior to use. After it has cooled down, the crucible was weighed (A). 50 g of sludge was placed in the crucible and the weight (B) was taken again before it was heated at 300 ^{o}C for 24 hour. The crucible filled with sludge that was heated was then weighed again (C) after it has cooled to room temperature.

To determine the percentage of volatile solids, the crucible was heated at 550 ^{o}C for 1 hour. Then, it was left to cool down at ambient temperature, before its weight was recorded (D). 10 g of the sample was placed in the crucible and weighed (E). Consequently, the crucible containing the sample was heated in a furnace for 1 hour at 300 ^{o}C. In the next 4 hour, the temperature was raised to around 550 ^{o}C. The sludge sample was then cooled and weighed (F). The percentage of total solid (% TS) and volatile solid (% TV) can be calculated using the following equations (equations 2.2 and 2.3);

$$\% \text{ TS} = \frac{B - C}{B - A} \times 100\ \% \qquad (2.2)$$

$$\% \text{ TV} = \frac{F - E}{E - D} \times 100\ \% \qquad (2.3)$$

2.4.4 Aqua Regia Digestion for Metal Analysis of Sludge

Dried sludge (2.0 g) was placed in a round bottom flask (100 mL) and 2 to 3 mL distilled water was added to form slurry. A 20.0 mL Aqua Regia solution (HCl-HNO_3 mixture in ratio of 3:1) was subsequently added to the same round bottom flask and then left covered overnight at room temperature. The mixture in the flask was refluxed using a 40 cm condenser for 2 hours and later allowed to cool at room temperature. Prior to reflux, 1-2 pieces of boiling rock were added to the round-bottom flask. The condenser was rinsed with 30.0 mL distilled water into the flask. Thereafter, the solution was filtered through a Whatman No. 2 filter paper and the filtrate was collected in a 100 mL graduated flask. The filtrate was subjected to subsequent elemental analysis using AAS.

2.4.5 Determination of Phosphorus

The determination of phosphorus, P was carried out based on the determination of ascorbic acid. The sludge sample (1.0 g) was placed in a beaker (50 mL), 2 to 3 mL of distilled water and 0.25 of sodium persulfate was then added in the sludge. The mixture was agitated and 10.0 mL of H_2SO_4 (5.25 N) was added before the samples were heated

slowly for 30 minutes. As the samples were heated the beaker was covered to prevent moisture from being released. It was then cooled to room temperature. After that the sample was filtered in a 50 mL volumetric flask using Whatman No 2 filter papers. 10 mL of NaOH (5 N) was added to the solution and the volumetric flask was then topped up with distilled water. Phos Version 3 reagent (0.25 g) was finally added before the phosphorus was determined using UV spectrophotometer Hach DR 4000U at a wavelength of 800 nm.

2.4.6 Total Kjeldahl Nitrogen Analysis

1.0 g of sample was weighed into a Kjeldahl flask. 5.00 mL of digestion reagent (potassium sulphate and sulfuric acid mixture in the ratio of 1:1), 10.00 mL copper sulphate, 10.00 mL hydrogen peroxide and 2 drops *n*-octanol were added into the Kjeldahl flask which was later heated using a heating mantle and the mixture digested for about 2 hours. The flask was then removed and left to cool at room temperature 75.00 mL of distilled water was later added into it. An excess of NaOH (10.0 M) was also added into the flask. A 250.0 mL titration flask containing 25.0 mL of 4 % boric acid as an indicator was placed with the tube from the condenser extending below the surface of the trapping solution**.** The titration flask was then attached to the distillation unit. A 100-125 mL steam distillate was collected. The titration flask was removed from the unit and the condenser tip rinsed with water. Trapping solution was titrated with 0.01 M H_2SO_4 to a neutral gray endpoint. The volume of acid (V_A) required was recorded. The titrate involving the reagent blank (V_B) was similarly performed. The color change observed was from green to gray to purple colour. The percentage of TKN was obtained using the formula as follows;

$$\% \text{ TKN} = \frac{14.01 \times (V_A - V_B) \times [H_2SO_4] \times 100\ \%}{\text{Mass of sample (g)} \times 10} \qquad (2.4)$$

2.5 Procedure

The procedures that were described below comprised of standard solution preparation, calibration curves and preparation of blank sludge sample.

2.5.1 Preparation of Standard Mixture Solution

100 µL of each of the seven standard PCB (10 µg/mL) were transferred using micropipette into a sample vial (20 mL), then 300 µL *iso*-octane was added to the mixture to produce a PCB standard mixture of 1.0 µg/mL. The solution was then refrigerated prior to analysis. The preparation of PAH and OCP standard mixtures has been described in detail by Chong (2002).

2.5.2 Calibration Graph

Each individual standard PCB was injected into the GC-ECD using the GC conditions as described in Section 2.2.2. The PCB standard mixture (1.0 µg/mL) was diluted to give a concentration of 0.5, 0.2, 0.1 and 0.05 µg/mL. The resultant solutions were used for the construction of calibration curves. Each solution was added with 50 µL PCB 35 as the internal standard. For calibration of PAH and OCP solutions, please refer to Chong, (2002) thesis.

2.5.3 Blank Sludge Samples

Blank sludge samples used for analyte recovery studies were prepared in the laboratory. Approximately 10.0 g of the sludge samples were initially subjected to exhaustive Soxhlet extraction for the removal of organic contaminants, enabling it to be used as blank sludge samples.

2.6 Conventional Soxhlet Extraction

Sludge sample (1.0 g) was placed in an extraction thimble together with 1.0 g of anhydrous sodium sulfate. Soxhlet extraction was performed using 160 mL acetone-dichloromethane (1:1) mixture and the extraction was carried out for 24 hours. The soxhlet extract was filtered through a Whatman filter paper. The filtrate was concentrated to about 1-2 mL using a rotary evaporator. The elimination of sulfur was carried out using copper powder (0.5 g), which was previously activated using concentrated HCl. After that, the extracts were clean-up using silica gel column. The final determination by GC-ECD was then carried out.

2.7 Supercritical Fluid Extraction

The sludge sample was filled into the SFE extraction vessel (5.0 mL) and spiked with 100 μL of the PCB mixture (1.0 μg/mL). The spiked sludge sample was exposed at room temperature in order to evaporate off the solvent. Prior to extraction, the SFE oven system was preheated to equilibrate the system.

The sludge was then extracted with supercritical carbon dioxide using the SFE/SFC Jasco instrumentation (Section 2.2.3). Pure carbon dioxide was selected for

SFE for preliminary extractions of the blank sludge. Analyte collection was performed through a stainless steel outlet in a solvent trap. The extract was then evaporated to near dryness and 100 μL *iso*-octane was added just prior to injection into GC-ECD.

The following parameters were investigated for optimizing analyte recoveries using SFE (i) extraction temperature, (ii) extraction pressure, (iii) percentage of methanol as modifier, (iv) flow rate, (v) solvent trapping and (vi) extraction time. The extraction efficiency was assessed by comparing the amount of each analyte recovered from the extract with its initial concentration in the sample. The percentage extraction or recovery can be calculated by using equation 2.5 as follows;

$$\% \text{ Recovery} = \frac{\text{Concentration of analyte in extract}}{\text{Concentration of analyte in spiked sample}} \times 100\% \qquad (2.5)$$

2.8 Subcritical Water Extraction

The extraction cell was half filled with the sample (0.5 g). After assembling the extraction cell in the oven, the oven was brought up to the working temperature. The high-pressure pump was switched on, while valve 1 was opened, and valve 2 was kept closed. The water was pumped into the extraction vessel until the pressure reached 50 bar. The high-pressure pump and valve 1 was closed switched off a one minute static extraction. After that, the pump was switched on again with valve 1 opened, while valve 2 was opened in order to maintain pressure at 50 bar. The outlet tubing was circulated through an ice water bath and extract collected in a vial within a 10 minute duration using 5.0 mL dichloromethane as the trap solvent. The water layer from each extract was removed using liquid-liquid partitioning, which was performed twice. The two organic solvents were then combined for GC-ECD analysis. Solutions were evaporated to near dryness, and 100 μL *iso*-octane was added to the final extract for GC analysis.

The parameters investigated for optimization of SWE recoveries were (i) extraction temperature, (ii) water flow rate, (iii) solvent trapping, and (iv) extraction time. The extraction efficiency was assessed by comparing the amount of each analyte recovered from the extract with its initial concentration in the sample. The percentage extraction or recovery can be calculated by using equation 2.5.

2.9 Extract Clean up

The SPE extract clean up was carried out using SPE Si cartridge mounted on a SPE vacuum manifold that was connected to a vacuum pump. Cartridges were conditioned with 5.0 mL of *n*-hexane-dichloromethane (80:20) and 10.0 mL of *n*-hexane before use. Extracts collected were applied to the SPE cartridge and eluted with 10.0 mL of hexane. The eluate was reduced to near dryness under a gentle stream of nitrogen gas and reconstituted with iso-octane for subsequent GC-ECD analysis (Folch *et al.,* 1996).

For the preparation of silica gel column, silica gel was activated at 130 ^{o}C for 8 hour. After it has been left to cool down, 6.0 g of silica gel was weighed into a glass container (Jang and Li, 2001). Then, 40 mL dichloromethane was added to produce silica gel slurry. The glass column for the clean-up and was 300 mm long with 11 mm i.d. It was fitted with glass wool and stopcock at the bottom of the column and pre-filled with dichloromethane before filling it up with the silica gel slurry. Anhydrous Na_2SO_4 (approximately 1.0 g) was added to the top of the silica gel column. The column was pre-eluted with 40 mL of hexane before sample application. Extracts collected were applied on top of the silica column and eluted with 20 mL of hexane in three fractions. The eluate was reduced to near dryness under a gentle stream of nitrogen gas and reconstituted with iso-octane for subsequent GC-ECD analysis.

2.10 Analysis of Real Sludge Samples

Real sludge samples were extracted using the most favourable extraction procedure, SFE. The extraction and analytical procedure is outlined briefly in Section 2.6. GC-ECD analysis was performed for the sample extract, followed by co-injection and GC-MS for further confirmation.

CHAPTER 3

CHARACTERIZATION STUDIES OF SEWAGE SLUDGE

3.1 Introduction

This chapter will be focusing on the result on the characterization of sewage sludge in terms of the following parameters; (i) moisture content, (ii) pH of sewage sludge, (iii) percentage of total solids and volatile solids, (iv) heavy metals determination (Zn, Cu, Pb, Ni and Cr), and (v) determination of nutrient content in sludge (TKN, P and K).

3.2 Moisture Content

Sludge samples were collected as wet solids or in the slurry state. The moisture content of all samples is shown in the Table 3.1. The below results showed that the moisture content are above 60 % indicating high water content in all of the samples. The highest percentage of moisture was from TD with 90.11%, followed by TI (89.75 %), KIS (81.81 %), TY (72.19 %), LK (65.38 %), SA (61.63 %) and TS (37.52 %). TD, TY and TI samples have the higher water content due to the fact that the samples were taken straight from the oxidation pond. The other samples which were taken from the drying

beds of the treatment plants however KIS samples showed high moisture content. This drying bed was wet due to rain prior to sampling.

Table 3.1 The moisture content of sludge sample.

Sample Code	Percentage Moisture (% ± RSD)*
LK	65.53 ± 0.97
SA	61.38 ± 0.62
KIS	81.81 ± 1.53
TI	89.75 ± 2.05
TD	90.11 ± 3.14
TS	37.52 ± 0.86
TY	72.19 ± 0.82
SI	60.11 ± 3.12

* n = 3

Chong (2000) reported the moisture content of sludge collected in SA and LK were 81.64 % and 15.58 % respectively. The previous studies reported the sludge moisture content in the range of 30 % to 90 % (Stabnikova *et al*., 2004 and Wang *et al*., 2005).

3.3 pH of Sludge

The determination of pH value for sludge sample whether it is acidic, basic or neutral is another way to characterize sludge. The pH measurement was conducted by using a digital pH meter that was calibrated using standard buffer solution at pH 4.01,

7.00 and 10.01. pH measurement was based on hydronium activity in the sludge (Winegardner, 1995). Table 3.2 shows the pH of sludge sample in eight different areas selected for this study. The results showed that these samples have pH ranging from 4.3 to 6.6. Samples LK and SA showed moderate acidity compared to other samples that were less acidic. The acidity of sludge was primarily due to the source of the sludge. Thus, if the sludge were to be used for agricultural purposes, proper measures has to be taken for LK and SA sludge samples as the optimum pH for most arable crops is 6.5 on mineral soils and 5.5 on peaty soils (Winegardner, 1995).

As a comparison, the pH values of sludge reported from other studies were compared with the data collected. Comparison of value pH from other studies reported sludge pH values ranging from 6.30 to 6.85 (Fatiha *et al*., 2000 ; Stabnikova *et al.*, 2004 and Wang *et al.*, 2005).

Table 3.2: pH of sludge samples under study

Sample code	pH ± RSD*
LK	4.31 ± 0.21
SA	4.32 ± 0.33
KIS	5.67 ± 0.27
TI	6.00 ± 0.35
TD	6.68 ± 0.47
TS	5.21 ± 0.24
TY	6.32 ± 0.30

*n=3

3.4 Percentage of Total Solid and Total Volatile Solid

Weight loss percentage is referred to as the weight of the residue left after being dried at specific temperature. Table 3.3 below shows the percentage weight loss that consists of total solid obtained at 300 ^{o}C and total volatile solid obtained at 550 ^{o}C (See Section 2.4.3). The percentages of total solids are ranged from 41 % to 76 %, except for TS, that has a low total solid of 41.97 %.

As to volatile solid it refers to the removal of organic compound from sludge when the sample was oxidized at 550 ^{o}C. This temperature can be ascribed to the dissociation and rupture of aromatic structure (Avanni and Gennaro, 1994). The organic matter in the sludge is being referred to as the percentage of total volatile solids. The determination of volatile solids is a useful method in water treatment as it gives the percentage of organic matter in the solids of the sludge.

Table 3.3 Percentage weight loss

Sample Code	Percentage Weight Loss (% ± RSD)*	
	300 ^{o}C (TS)	550 ^{o}C (TV)
LK	70.94 ± 2.11	19.29 ± 3.14
SA	64.69 ± 2.54	26.81 ± 4.71
KIS	67.84 ± 1.57	27.25 ± 1.77
TI	76.83 ± 3.12	12.31 ± 2.20
TD	72.11 ± 2.71	15.11 ± 3.12
TS	41.94 ± 4.11	20.29 ± 4.14
TY	65.26 ± 3.52	22.81 ± 3.72

*n=3

The overall result for percentage of total volatile solid is in the range of 10 % to 27 %. This shows that the samples are stable chemically compared to the non-stable sludge, which consists of 75 % to 85 % of volatile solids (Avanni and Gennaro, 1994). Sample KIS showed the highest amount of volatile percentage with 27.25 % and sample TI with the least percentage of total volatile solid (12.31 %).

3.5 Nutrient Content in Sludge

There are sixteen nutrient elements that are essential for the growth and reproduction of plants. Plants obtain the three most abundant nutrients carbon, hydrogen and oxygen from water and air. The other 13 elements are divided into three categories: primary, secondary and micronutrients. Nitrogen (N), phosphorus (P) and potassium (K) are primary nutrients that are needed in fairly large quantities compared to the other nutrients. Calcium (Ca), magnesium (Mg) and sulfur (S) are secondary nutrients, which are required by the plant in lesser quantities but are no less essential for good plant growth than the primary nutrients. Zinc (Zn), manganese (Mn), iron (Fe), boron (B), copper (Cu), molybdenum (Mo) and chlorine (Cl) are micronutrients, which are required by plants in very small amounts (Havlin *et al.*, 1999).

Nitrogen that is organically bound in the trinegative state is defined as organic nitrogen, which includes compounds like peptides, proteins, and nucleic acids (Havlin *et al.,* 1999). Meanwhile, the inorganic nitrogen species include compounds like ammonia, nitrite, and nitrate. The Total Kjeldahl Nitrogen (TKN) test is widely used to determine organic compounds in the trinegative oxidation state and ammonia nitrogen in a waste stream. This test converts amino nitrogen to ammonium through a digestion performed at 380 °C in the presence of sulfuric acid. The exact time and temperature for optimum digestion depend on the contents of the sample.

Table 3.4 shows the result of total kjeldahl nitrogen in the samples. The percentage of nitrogen content in sludge ranged from 0.059 to 0.98 %. The lowest percentage of total nitrogen was from sample TD with 0.059 % while the highest percentage nitrogen was from sample LK sample with 0.98 %. Based on a previous work, similar result has been observed by Chong (2000). Fatiha *et al.,* 2000 reported the total nitrogen in sludge of around 2.15 %. In typical common fertilizer, the total nitrogen content is in the range of 3.5 to 6.4 %, which is sufficient for the plant growth (Havlin *et al.,* 1999).

Table 3.4 Percentage of TKN in sludge sample

Sample code	This Study (%)	Previous Study (%)*
LK	0.98	0.69
SA	0.75	0.76
KIS	0.73	NA
TI	0.19	NA
TD	0.059	NA
TS	0.82	NA
TY	0.33	NA

* Chong (2000), NA= Not available

The phosphorus (P) and potassium (K) content in sludge samples are shown in Table 3.5. From the table, it is shown that P content in sludge is 0.05 to 0.15 %. The lowest percentage of P was observed from the sample KIS, LK and TY ranged from 0.04 to 0.09 % whereas the highest was from sample TI (0.15 %). Fatiha *et al.,* 2000 reported the phosphorus content in sludge of only 0.004 % while other studies reported the percent of phosphorus in the sludge to be in the range 0.2 to1.9 % (Stabnikova *et al.,* 2004; Wang *et al.,* 2005). Most of the phosphorus in sewage sludge is in an inorganic form, the content customarily being expressed in term of P_2O_5. The introduction of

synthetic detergents during household activities may increase the amount of phosphate in sludge, most packaged detergents containing 20 to 40 % phosphate (Wilson and Jones, 1994). Unlike nitrogen, phosphate is not leached from the soil by rain and if soil contains an excessive amount, it does not appear to affect adversely the growth of plants.

For potassium content, the entire sludge samples contained 0.12 to 0.16 % of K. Sample TI showed the highest amount of potassium content (0.16 %). Compared to the previous research by Chong (2000), all sludge samples showed similar % K in this study. Reports by Fatiha *et al*., (2000), Stabnikova *et al.,* (2004) and Wang *et al.,* (2005) indicated the potassium content in sludge to be in the range of 0.6 to 1.9 %.

Table 3.5 Percent phosphorus and potassium in sludge

Sample code	P (%)	K (%)
TD	0.11 ± 0.05	0.13 ± 0.03
SA	0.10 ± 0.03	0.15 ± 0.02
KIS	0.05 ± 0.03	0.14 ± 0.02
LK	0.05 ± 0.01	0.12 ± 0.02
TI	0.15 ± 0.02	0.16 ± 0.03
TS	0.12 ± 0.02	0.12 ± 0.01
TY	0.09 ± 0.01	0.13 ± 0.04

n=3

Several countries have yet to adopt a practical, economical and acceptable approach in managing and disposing of sewage sludge. The use of sewage sludge as an agricultural fertilizer is at the center of an intense debate (Hing *et al*., 1998). Typical sewage sludge may contain 3 % N, 2.2 % P and 0.3 % K (Lasa *et al.,* 1997). Parkpian *et al.,* (2003) reported the sources of N, the combination of urea 33 % N and sewage sludge 67 % N resulted in highest grain yield. Parkpian *et al.,* (2003) indicated that a

combination of urea and quality sewage sludge could be used as a fertilizer nitrogen source for rice, without risks associated with toxic heavy metals.

In this study, it was found that the NPK content in sludge was lower compared to that of commercial fertilizer. This sludge may be suitable as added fertilizer or for plant growth that require only low amounts of NPK content.

3.6 Determination of Heavy Metal

The concentration of five heavy metals (Cu, Zn, Ni, Pb and Cr) in sludge is shown in Table 3.6. Results showed that sludges near the industrial area have higher concentration of metal compared to other. This was predicted as metals were used more in industries.

For determination of Cu, LK samples showed the highest concentration with 382.5 mg/kg. It was followed by KIS and TS samples with 240.0 and 151.1 mg/kg. Sludge from SA has also high concentration of Cu with 150.0 mg/kg. For determination Zn, KIS sample has the highest concentration of 1467.2 mg/kg and was followed by TY and TS. All sludge samples have low concentration of Ni below 50.0 mg/kg. However two sludge samples have high concentration of Pb; which is TY and TS samples. For Cr, all the samples showed level result below 100 mg/kg.

Domestic sources account for the largest amounts of certain important elements, particularly of Zn and Cu. Critchley and Agg (1986) reported that 62 % of Cu entering the sewer system was from domestic sources compared to 5 % from road run-off and only 3 % from the industries. The presence of Zn in the household (sample TS and TI) discharges is explained through the corrosion of both galvanized iron domestic plumbing system. The input Zn in WTP could be attributed to domestic activities including clothes and dish washing, and from product care such as shampoos. Meanwhile lead could

originate from engine repair and mechanical activities. Water or rain run off is an most important diffuse source of lead entering the sewer system arising from the contributions of lead from car exhaust (Critchley and Agg, 1986).

Chromium in sewage sludge may be derived mainly from industrial wastewater, such as the waste from the plating and electronic industry. It is normally present in a form of insoluble trivalent in digested sludge digested sludge and in any case where the presence of soil organic matter appears to bring about a rapid reduction to this form. Meanwhile for the nickel content in sewage sludge, it may originate from the industrial wastewater such as electroplating and chemical industry (Critchley and Agg, 1986).

Comparison with USEPA limit values for heavy metals in this study shows none of the heavy metals found were over the maximum limit. Another study by Rosenani *et al.,* (2004) reported that, the heavy metal (Pb, Cd, Cu, Mn and Ni) concentration in the sludge, except for Zn are below the European Union Maximum permitted level in sludges. These results were supported by the work of Pereira and Kuch (2005) reported that the sludge generated by urban wastewater treatment plant is below the maximum established limit. Zufiaurre *et al.,* (1998) also reported that sewage sludge from Zarogaza none of heavy metals found over the maximum limit.

Such low concentration of heavy metal from municipal sludge yielded in this study render the sludge suitable for agricultural or for added fertilizer purposes. This is because it may provide a valuable contributor of microelements, which have a positive effect on soils and organisms living in them.

Table 3.6 Concentration of heavy metal in sewage sludge

Heavy Metal / Sample Code	Concentration (mg/kg) ±SD*				
	Cu	Zn	Ni	Pb	Cr
LK	382.5±10.6	877.5±38.8	41.5±2.1	127.2±0.3	94.5±0.7
SA	150.0±10.1	86.5±4.2	8.2±2.4	153.7±2.4	67.0±5.6
KIS	240.0±11.1	1467.2±21.3	7.8±1.6	58.8±1.2	23.8±0.5
TI	40.0±7.0	213.5±24.7	10.5±0.2	34.7±3.8	30.2±3.1
TD	32.2±1.7	88.5±5.6	9.2±0.3	9.0±1.4	14.7±1.0
TY	72.6±5.2	1233.3±25.1	45.2±4.2	261.1±10.6	71.5±8.2
TS	151.1±10.1	1016.6±23.3	9.7±1.1	231.3±9.8	44.3±7.1
#Limit	4300	7500	420	840	600

#United State Environmental Protection Agency (USEPA)

*n=3

3.7 Summary

As a conclusion for this chapter, the characterization of sewage sludge sample in this study has been carried out. The sludge samples showed high water content (37 to 90 %) and for pH values ranging from 4.3 to 6.6. All samples have to be chemically stable as reflected from the percentage of total solid and volatile solid.

From the determination of nutrient in the sewage sludge samples, it is shown that the percentage of the TKN, P and K in sludge were in the range 0.059 to 0.98 %, 0.05 to 0.15 % and 0.12 to 0.16 %, respectively. This indicated that the sewage sludge has nutrient content much lower than the commercial fertilizer. Heavy metal studies showed

that metal content in municipal sewage sludge samples were below the USEPA limit. Low concentration of heavy metals in the sewage sludge samples indicates suitability for agricultural use since they are valuable contributor of microelements, which have a positive effect on soils and organisms.

CHAPTER 4

RECOVERY STUDIES OF PCBs

4.1 Introduction

This chapter presents the discussion on the GC-ECD separation of standard PCB mixture, calibration graph and limit of detection. The optimization of SF-CO_2 and SWE methodologies for the analysis of PCB in sewage sludge will also be thoroughly discussed.

4.2 Gas Chromatographic Separation of PCB mixture

Seven PCBs of common occurrence in sewage sludge were chosen for determination with GC-ECD using a HP-5MS capillary column. The target analytes were PCBs 28, 52, 101, 118, 138, 153 and 180. All seven PCBs were successfully separated, with a total time of less than 30 minutes. The gas chromatographic resolution of seven PCBs is illustrated in Figure 4.1.

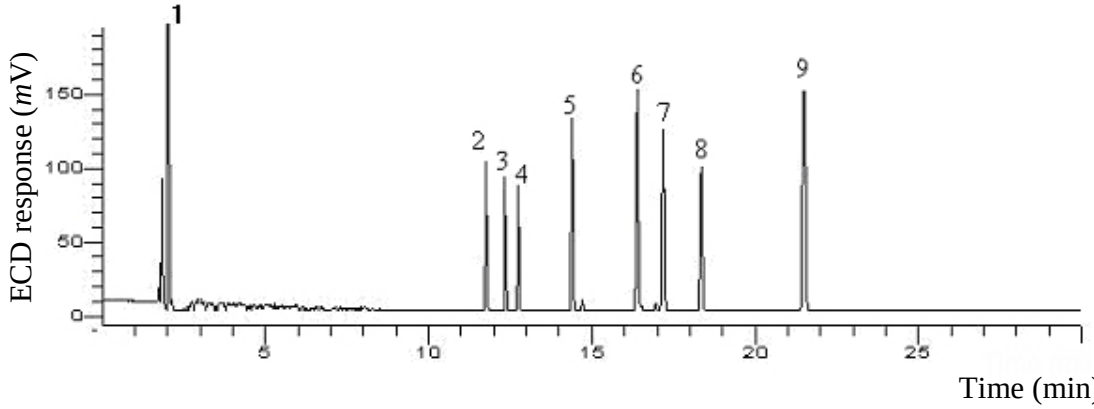


Figure 4.1 GC-ECD separations of the PCBs using a HP-5MS capillary column. GC Conditions: Injection port temperature at 280^{o}C and detector temperature at 290 ^{o}C. Temperature programming from 200^{o}C (held for 3 minutes) then ramped to 230 ^{o}C (held for 3 minutes) at a rate of 5 ^{o}C/min and increased to 260 ^{o}C (held for 3 minutes) at a rate of 2 ^{o}C/min. Peak identity: (1) *iso*-octane (2) PCB 28, (3) PCB 52, (4) PCB 35, (5) PCB 101, (6) PCB 118, (7) PCB 153, (8) PCB 138 and (9) PCB 180.

Table 4.1 shows the capacity factors of the PCBs. From the table, it is shown that PCBs were eluted according to the increase in their molecular weight and chlorine atom position as well as their boiling points.

Table 4.1 Capacity factors of the standard PCBs

PCB	Molecular Formula	t_r (min)	* k'	Molecular weight (g/mol)
PCB 28	2, 4, 4' – trichloroBp	11.71	4.8	257.5
PCB 52	2,2', 5, 5' - tetrachloroBp	12.33	5.1	292.0
PCB 101	2, 2', 4, 5, 5' – pentachloroBp	12.71	5.3	326.5
PCB 118	2, 3', 4, 4', 5 – pentachloroBp	14.32	6.1	326.5
PCB 153	2, 2', 4, 4', 5, 5' – hexachloroBp	17.17	7.5	361.0
PCB 138	2, 2', 3, 4, 4', 5' – hexachloroBp	18.32	8.1	361.0
PCB 180	2, 2', 3, 4, 4', 5, 5' – heptachloroBp	21.41	9.7	395.5

* $k' = (t_r - t_o) / t_o$, t_o = 1.9 min, Bp = Biphenyl, t_r = Retention time

4.3 Calibration Graph of Standard PCBs

A calibration graph was carried out for each PCB studied. A set of standard PCB mixture with different concentrations was prepared (0.1, 0.25, 0.5 and 1.0 µg/mL). A fixed volume of PCB 35 (50 µL of 10 ppm) was added as an internal standard into each of the solution prepared. PCB 35 was selected as an internal standard since it is not commonly found in sewage sludge.

The calibration graph for each PCB was prepared by plotting the ratios of peak area of PCB relative to the peak area of internal standard (IS), versus the concentration of the PCBs. Four calibration points were used for each analyte and the calibration curves produced were used for further quantitative analysis. Triplicate injections for each standard mixture were carried out. A linear calibration graph was produced for each PCB with good correlation coefficients ranging between 0.9907-0.9980 (Figure 4.2).

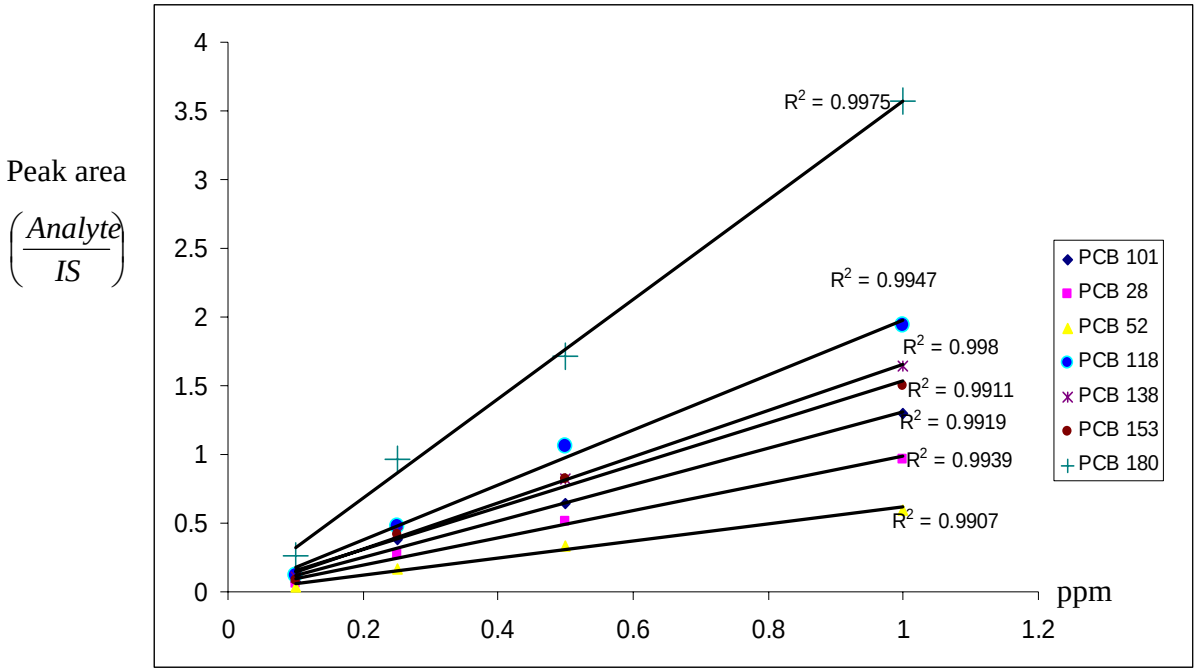


Figure 4.2 Calibration curves for PCBs under study.

4.4 Limits of Detection

In order to determine the sensitivity of the technique, the limit of detection (LOD) of each PCBs was determined by the analyte signal three times the background signal. Detection limits for the PCBs showed in Table 4.2. The LOD for the seven PCBs show were generally higher compared to other study (Punín and Lage, 2006). The difference in LOD could be due from the different type of capillary column used as well as the sensitivity of the ECD of detection system. In this study the column used was an HP-5MS whereas Punin and Lage employed a BP5 capillary column. The value of LOD in this study is applicable for analyses of real samples because PCBs level in sewage sludge are generally in the range between 1.0 to 100 mg/kg (Hing *et al*., 1998).

Table 4.2 Limit of detection for PCB

PCB isomer	Detection Limit (µg/mL)	
	This work	Other Study*
PCB 28	0.030	0.008
PCB 52	0.048	0.006
PCB 101	0.026	0.006
PCB 118	0.028	0.0006
PCB 138	0.033	Nr
PCB 153	0.027	0.010
PCB 180	0.016	0.007

*Punín C. and Lage Y., (2006), Column : BP5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film) (SGE, Australia)
Nr-Not reported

4.5 PCB Analysis Using Soxhlet

Extractions of PCBs for sludge samples were initially conducted using the conventional soxhlet extraction. However, the soxhlet extracts required further extract clean-up prior to GC-ECD analyses. Thus, it was desirable to employ an appropriate extract clean-up procedure for PCB sludge analysis.

4.5.1 Optimization of Extract Clean-Up

In order to optimize extract clean-up using silica gel, recovering studies of PCBs from silica gel column and silica SPE cartridges were performed.

The procedure on the extract clean-up using silica gel procedure is as mentioned in Section 2.9. Figure 4.3 shows the elution of target PCB at cumulative recovery at each elution volume. As shown in Figure 4.3, higher molecular weight congeners like heptachlorobiphenyl (PCB 180) and hexachlorobiphenyl (PCB 153 and 138) were eluted more quickly compared to the lower molecular weight congeners. Jang and Li (2001) also reported similar observations in their study, the higher molecular weight congeners like decachlorobiphenyl and nonachlorobiphenyl were found to elute more quickly compared to the lower molecular weight congeners.

The higher molecular weight PCBs (PCB 138, 153 and 180) were completely eluted out of the column with 40 to 50 mLs of the eluting solvent. By contrast, the lower molecular weight PCB required 50 to 60 mL of solvent for complete elution. The result of this study are in good agreement with that reported by Martinez *et al.*, (2005) who found that the best results for PCB extract of clean-up were obtained using silica and *n*-hexane as the solvent elution.

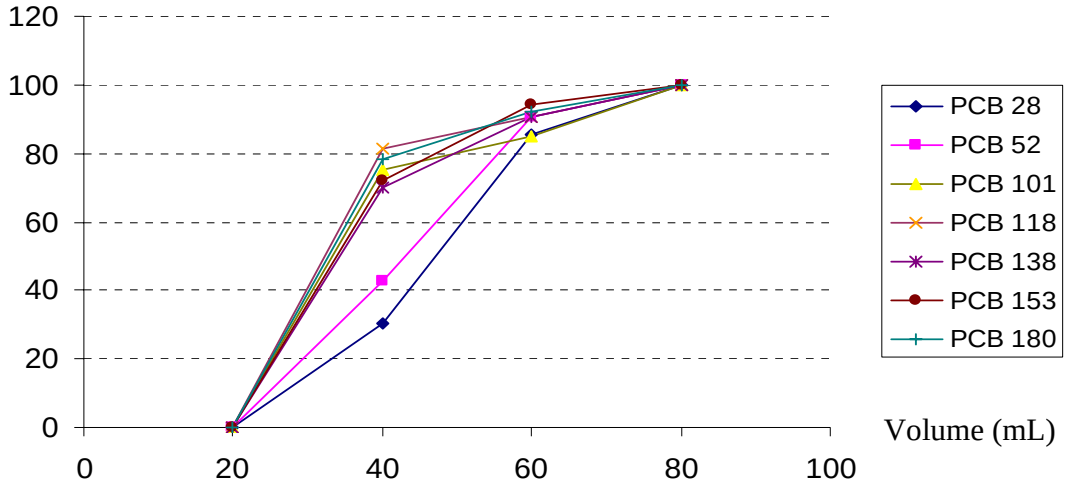


Figure 4.3 Elution of PCB at cumulative recovery at each elution volume.

The procedure on the extract clean-up using silica SPE cartridge procedure is mentioned in Section 2.9. Table 4.3 shows the recovery of PCB using SPE silica ranged from 72.7 to 84.2 %. The results showed that both procedures gave quantitative recoveries of PCB, however silica gel column was evidently more efficient than the SPE silica cartridge. Hence for this study, the silica gel column was employed for extract clean-up.

Table 4.3 PCB recoveries using SPE silica and silica column

PCB isomer	Recovery (%), ± RSD*	
	SPE silica	Silica column
PCB 28	72.7 ± 3.6	85.4 ± 2.1
PCB 52	78.6 ± 5.0	90.5 ± 4.2
PCB 101	73.7 ± 5.7	84.9 ± 3.2
PCB 118	81.8 ± 3.8	90.7 ± 2.5
PCB 138	82.1 ± 2.7	90.9 ± 3.1
PCB 153	82.3 ± 1.6	94.1 ± 4.3
PCB 180	84.2 ± 4.9	92.4 ± 5.2

*n=2

4.6 SFE of PCBs

SFE was initially considered as the new extraction technique of choice for the extraction of PCBs for sludge. In order to assess the efficiency of the supercritical CO_2 extraction of PCBs, recovery studies of PCBs were conducted using spiked sludge samples. Recovery studies were not performed using Certified Reference Material (CRM) due to the unavailability of commercially CRM for sludge, so these studies were conducted using blank sludge.

To obtain the optimum SF-CO_2 conditions for the extraction of PCBs in sludge sample, several parameters such as extraction temperature, extraction pressure, composition of extractant modifier, CO_2 flow-rate, solvent trapping and extraction duration time were optimized.

4.6.1 Effect of Extraction Temperature

The effect of extraction temperature was initially observed by varying the extraction temperature (40 to 70 ^{o}C) while keeping the other parameters constant (extraction of 30 minute, CO_2 flow rate of 2.0 mL/min, extraction pressure of 200 bar and analyte collected in methanol as the trap solvent). The recoveries of seven PCBs mixture (spiked at 1.0 mg/L) were investigated.

As shown in Figure 4.4, the PCB recoveries generally increase as the temperature increases. At an extraction temperature of 60 ^{o}C, all PCBs gave over 80 % recoveries with the exception of PCB 28 and PCB 52. On further increment to 70 ^{o}C, recoveries of most PCBs were not significantly different. The results of this study is in support of previously reported work which indicated that pure CO_2 at high temperature could yield high recoveries of many semi-volatile pollutants (Langenfeld *et al.*, 1993; Yang *et al.*, 1995; Kreuzig *et al.*, 2000 and Sporring *et al.*, 2005). The reproducibility of

extraction temperature on SF-CO_2 was found to be comparatively good with all samples with RSD below 7.3 % (Appendix B). Based on the above results, the optimum temperature was taken as 60 ^oC.

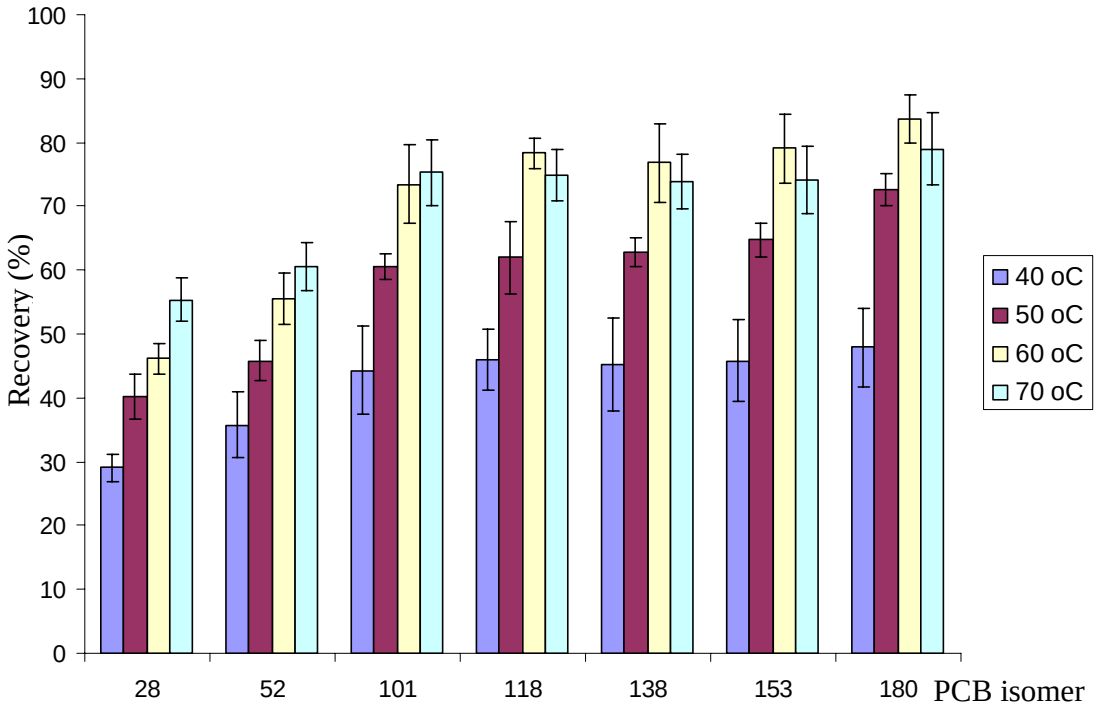


Figure 4.4 Effect of extraction temperature on the SFE recovery of the spike sludge using pure CO_2 at 2.0 mL/min with 30 min extraction and extraction pressure of 200 bar.

The effect of extraction temperature is complex and there is no simple theory that could explain on how temperature affects a given SFE process. Taylor, (1996) proposed a combination of the effect on thermodynamic and dynamic properties. Improved recoveries using high temperature extractions could be contributed by two factors (Castro *et al.*, 1994; Taylor, 1996). Firstly, high temperature can produce higher vapor pressure of the analytes, thus it will increase the analytes solubility despite the decrease in the density of CO_2 at high temperature. Secondly, a certain amount of energy is required to desorb the analyte from sludge. Hence, an increase of the extraction

temperature can provide more energy to the extraction system. This will also increase the rate of the desorption process. The temperature may also affect the diffusion as well as the mass transfer behavior. Extractions at high temperature may change the pores and increase contact area of sludge with CO_2.

Langenfeld *et al.*, (1993) reported that the temperature effect on SFE became important only when the temperature was increased from 50 ^{o}C to 200 ^{o}C. The positive effects of increasing temperatures, despite decreasing densities was demonstrated by Bjorklund *et al.,* (1999) for the extraction of PCBs. Zhu and Lee (2002) reported recovery of 95 % using extraction temperature of 120 ^{o}C, while no further improvement was obtained when the temperature was increased to 140 ^{o}C. However in this study, an extraction temperature above 70 ^{o}C was not utilised due to the maximum upper temperature of the oven being at 80 ^{o}C.

4.6.2 Effect of Extraction Pressure

Using the blank sludge as a model sample matrix, the PCB recoveries were studied over the pressure ranging from 100 to 400 bar by 100 bar, increments at a constant extraction temperature of 60 ^{o}C within 30 min extraction. The maximum operating pressure of the back-pressure regulator for the SFE system used was 500 bar. The effect of pressure on the extraction efficiency of PCBs is shown in Figure 4.5. The best optimum pressure for the extraction of PCB from sludge was found to be 200 bar which yielded the recoveries of PCB 101 to PCB 180 in the range of 70 to 81 % but for PCB 28 and PCB 52 only yielded 45 and 55 % respectively.

Generally when extraction pressure increases from 100 to 400 bar, the density of CO_2 also increases. When the extraction pressures were increased up to 300 bar it will cause a large decrease in the extraction efficiency for all PCBs except for PCB 28 and PCB 52. Further increase of pressure (400 bar) gave reduced recoveries of all PCBs.

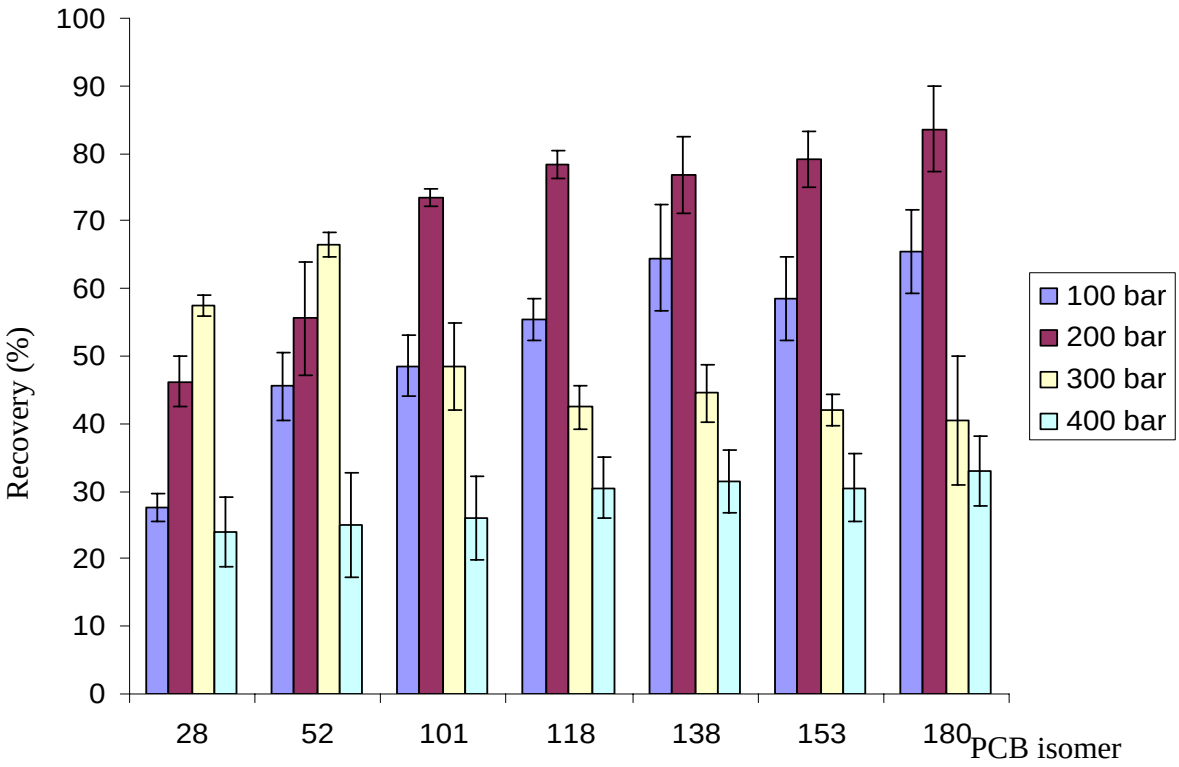


Figure 4.5 Effect of extraction pressure on the SFE recovery of the spiked sludge sample using CO_2 at 60^{o}C with 30 min and CO_2 flow rate 2.0 mL/min.

Thermodynamic properties such as solute solubility are necessary to understand the pressure effect on the extraction process (Langenfeld *et al.,* 1993). Velde *et al.,* (1992) reported that, the largest improvement in recovery was seen as when increasing the pressure increases from 150 to 200 bar, whereas only a slight increase in recovery is obtained from 250 to 200 bar. The solvating power increase proportionally with density, but the diffusion coefficients are inversely proportional to higher densities. The result is similar to that reported by Tong and Imagawa (1995) where the best optimal pressure for extraction of PCBs to be 200 bar.

At high pressures, the risk of coextraction to increase from the matrix is possible, so a moderate extraction pressure at 200 bar was adequate for extraction of PCBs in sludge. The reproducibility of extraction pressure on SFE was shown to be comparatively good with all samples with RSD below 9.6 % (Appendix B).

4.6.3 Methanol as Organic Modifier

Previous studies have indicated that the solvent power of pure dense CO_2 is not strong enough to extract persistent pollutant like PCBs from environmental samples. Moreover, the use of cosolvent is necessary to enhance the recoveries in SFE (Chen *et al.,* 1997; Abaroudi *et al.,* 1999). A few percent (1 to 15 %) of a polar modifier is often added to enhance the solubility of a more polar compound or to increase the ability of the supercritical fluid to better displace analytes from the matrix active sites. These modifiers are usually organic solvents that are added to the extraction fluid. They can be added using three different procedures (Castro *et al.,* 1994; Taylor, 1996). Firstly using direct addition of modifier to the sample, secondly premixed fluids were prepared by adding a modifier to the liquid CO_2 in the storage cylinder and lastly by using a separate modifier pump to pump in the organic solvent into the SF-CO_2 fluid.

Ethanol, acetone, methanol, ethyl acetate and some light hydrocarbons have been used in previous studies of the extraction of PCBs (Li *et al.,* 2003). Besides the chemical characteristics, the toxicology of the cosolvent is an important practical factor for the environmental waste decontamination. Therefore, a small amount of an organic solvent was added to the supercritical fluid to enhance the extraction of PCBs. The selectivity of methanol as a cosolvent in this study is based on the previous studies, which showed that methanol could be a good cosolvent for the extraction PCBs from solid samples (Chen *et al.,* 1997). The modifier was introduced as mixed fluids in the pumping system via a second pump. The composition of the methanol modifier was varied from 0 to 15 %.

Figure 4.6 shows the chromatogram of PCBs for SF-CO_2 extracts with and without modifier. The SF-CO_2 extract using 15 % methanol as modifier recorded more peaks on the chromatogram compared than the one without modifier. The color of the SF-CO_2 extract changed to pale yellow using methanol modified CO_2, which indicated that some components of sludge were extracted during SFE.

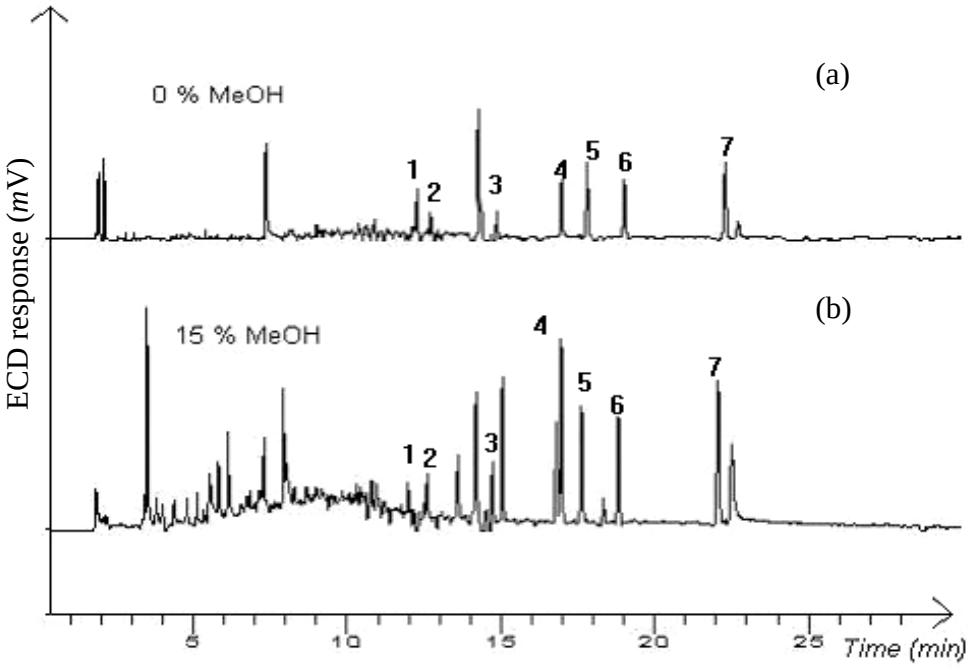


Figure 4.6 GC-ECD separations of PCBs from SF-CO_2 extract (a) without modifier (neat using SF CO_2) and (b) SF-CO_2 with 15 % methanol as modifier. Peak identity: (1) PCB 28, (2) PCB 52, (3) PCB 101, (4) PCB 118, (5) PCB 153, (6) PCB 138 and (7) PCB 180.

The effect of varying concentration of methanol as modifier for the SF-CO_2 of PCBs is shown in Figure 4.7. When the compositions of methanol increased from 0 to 10 %, the recoveries increased slightly for all PCBs. SF-CO_2 with 15 % methanol extraction gave the best overall extraction efficiency for all PCBs except for PCB 28 with only 49 % recovery. The increased percentage modifier does not give any significant contribution for PCB 28. The low percentage recovery of PCB 28 could be due to its lower molecular weight that may easily be lost during trapping system.

As the SFE results for pure CO_2 was compared with the result for modifier solvent, it was found that the extraction of PCBs from sludge was greatly improved with the addition of a small amount of methanol rather than using pure CO_2. These results were supported by the work of Li *et al.*, (2003), who used cosolvent to enhance recoveries in SFE. However, only PCB 28 showed the lowest recovery ranging from 42

to 50 % compared to other PCBs. The modifier addition also enhances the coextraction of contaminants to an extent equivalent to the soxhlet extraction and may need clean-up before the final quantification. In this study, the coextractants were found to not interfere with the analyte peaks. Hence, SFE of PCBs using modifier CO_2 did not require further extract clean-up.

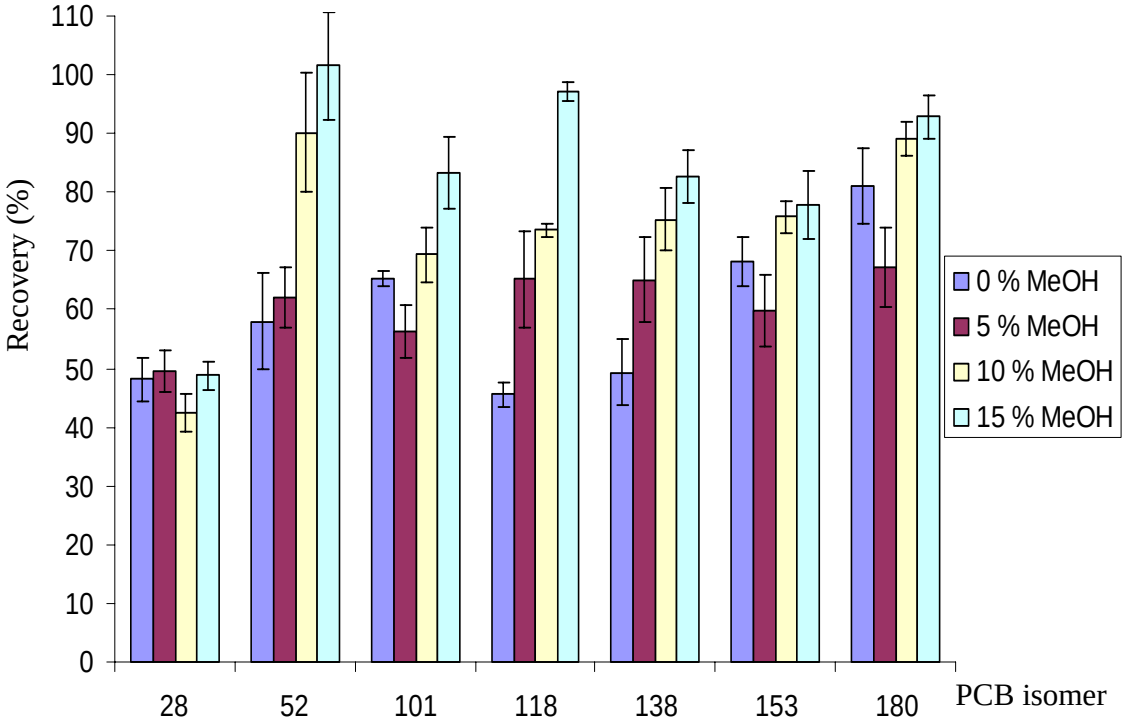


Figure 4.7 Effect of varying concentration of methanol as modifier on the SF-CO_2 recoveries of PCBs. SFE conditions: pressure 200 bar, SF flow rate 2.0 mL/min, 60 ^{o}C with 30 minute extraction time.

The enhancement of PCB extraction efficiency contributed by the addition of methanol could be mainly related to the interaction between methanol, PCB molecules, and sludge matrices instead of the increase of PCB solubilities during the extraction. These possibilities suggest that methanol improves desorption rates of weakly adsorbed PCBs and made portions of strongly bound PCBs being easily desorbed (Li *et al.*, 2003).

The mechanism of the cosolvent effect is rather complicated and several hypotheses have been proposed to explain the cosolvent phenomena. The cosolvent can weaken the analyte-matrix interaction such as Van Der Waals forces, electron acceptor electron–donor interaction and hydrogen bonding (Mijeong and David, 1999). It should be noted that the addition of large amounts of modifier will considerably change the critical parameters of the mixture (Crowther and Iteinon, 1985).

Based on the above results, the optimal percent of adding methanol to SF-CO_2 was taken at 15 % methanol. The reproducibility through the application of extraction modifier on SFE was shown to be comparatively good for all samples with RSD below 10.2 % (Appendix B).

4.6.4 Flow Rate

Flow rate parameter often determines the success or failure of SFE, and can also be varied to provide the information on dynamics aspect of the extraction process. It has been shown that if the flow of supercritical fluid is insufficient to wet the cell void volume, the effectiveness of the extraction would be reduced (Bowadt and Hawthorne, 1995). In fact, changing the extraction flow rate is a simple way to determine whether the extraction is limited by chromatographic retention, or limited by the kinetics of the initial desorption process (Bowadt and Hawthorne, 1995).

The effect of varying flow rate on the extraction efficiency of PCBs in spiked sludge was studied. The composition of flow rate was varied from 1.0 mL/min to 2.5 mL/min. From Figure 4.8, when the SF CO_2 flow rate was increased from 1.0 mL/min to 1.5 mL/min, recovery of all PCBs was found to be insignificant as it did not give any increase. This could be due to the low flow rate that is insufficient to force the analyte out from the matrix and therefore needed a longer time to complete the extraction. When SF-CO_2 was increased to 2.0 mL/min, the recovery of all PCB

increases, however further increment to 2.5 mL/min has caused a major decrease in the recovery all PCBs. High flow rate could decrease the recovery either by inducing an elevated pressure drop through the extraction cell or by increasing analyte loss during decompression of the fluid.

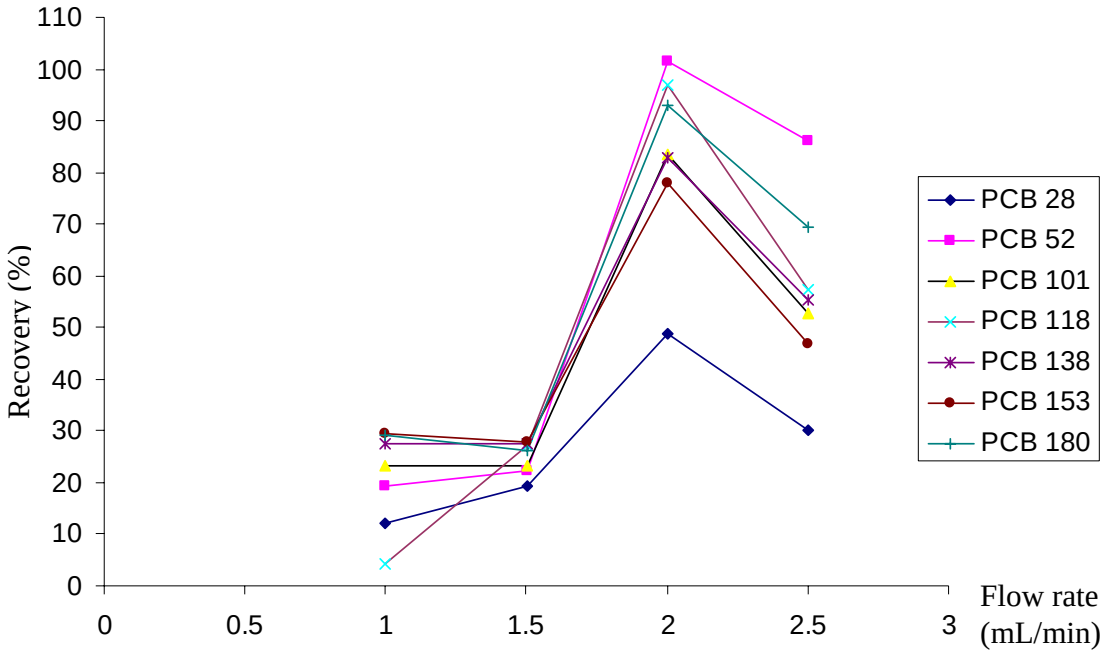


Figure 4.8 Effect of varying flow rate on the SFE recoveries PCBs. SFE conditions: pressure 200 bar, SF-CO_2 with 15 % methanol as modifier and 60 ^{o}C with 30 minute extraction time.

However, only PCB 28 showed the lowest recovery (50 %) at the optimum flow rate of 2.0 mL/min due to PCB 28 having lower molecular weight compared to other isomers. Based on the results in Figure 4.8, it was shown that the supercritical fluid extraction with flow rate 2.0 mL/min gave the best overall extraction efficiency for all PCBs. The data reproducibility using extraction flow rate on SFE was shown to be comparatively good with all samples with RSD below 9.2 % (Appendix B).

4.6.5 Effect of Trapping Solvent

As the analyte solubility depends on the SFE trapping solvent, three solvents of different polarity and boiling points, namely *iso*-octane, methanol and *n*-hexane were examined for their efficiency as trapping solvents for PCBs extraction. It was found that trapping solvents showed varying efficiency as shown in Figure 4.9. Generally, methanol and *n*-hexane were found to give better analyte recovery than *iso*-octane, although *iso*-octane showed higher recovery for PCB 28 and 118 compared to methanol and *n*-hexane. The reproducibility of extraction solvent trapping on SFE was shown to be comparatively good with all samples displaying RSD below 9.2 % (Appendix B).

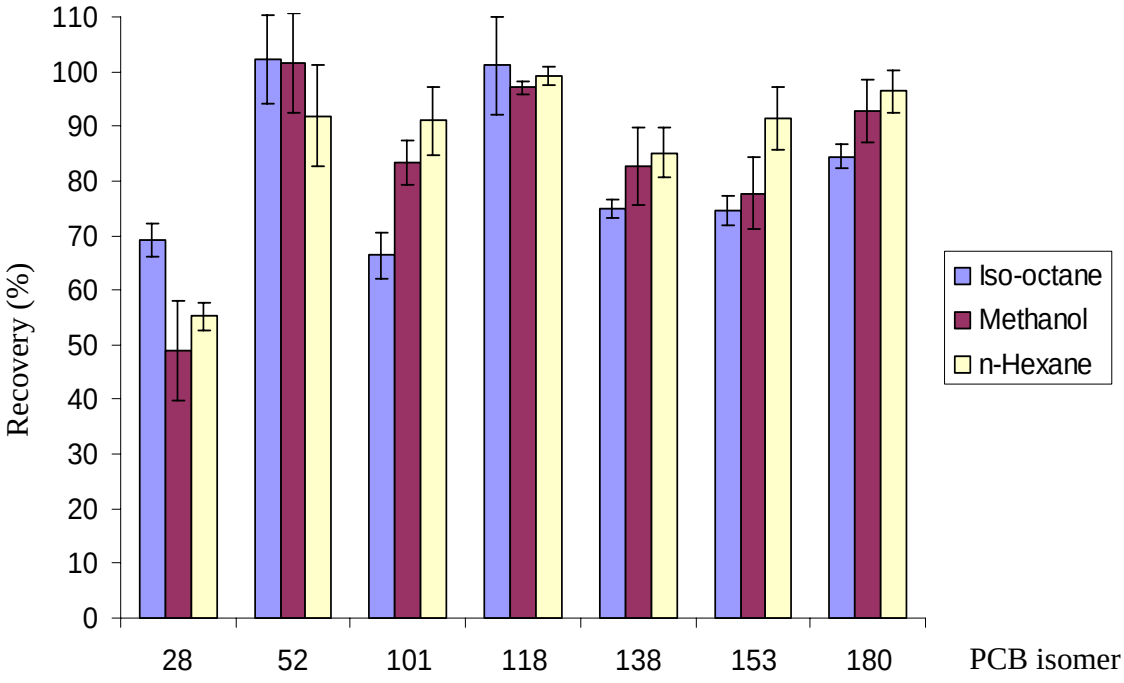


Figure 4.9 Effect of different solvent trapping on the SFE recoveries of PCB congener. SFE conditions: pressure 200 bar, temperature 60 ^{o}C, 15 % methanol as modifier and with 30 minute extraction time.

This result is in good agreement with a previously reported study by Velde *et al.*, (1992) where *iso*-octane yielded good recoveries for less chlorinated compounds.

n-hexane was selected as the trapping solvent in the later part of this work rather than methanol because methanol exhibited low recovery for PCB 28, 101 and 153. According to the results reported by Langenfeld *et al.* (1992), acetone was chosen as the trapping solvent. The volume of trapping solvent is important because the transition time of the CO_2 bubbles depends on the solvent volume. The volume of *n*-hexane in this study was 5.0 mL, as poor trapping in the solvent was observed when more than 10 mL was applied.

4.6.6 Duration of Extraction Time

All previous studies on the extraction were performed within 30 minutes. The effect of extraction period on the recovery of PCBs was studied using an online SFE-UV whereby the SFE system was modified by directing the extract through a UV detector fitted with a high-pressure flow cell and the outlet from detector was then connected to a back pressure regulator. The advantage of using an online SFE-UV system is that one could monitor the spectrum during extraction and could also predict the duration time of the extraction based on extraction profile in the spectrum. Figure 4.10 shows the extraction profile of online SFE-UV for PCBs at the optimum temperature 60 ^{o}C and pressure of 200 bar using 15 % methanol as modifier.

From the extraction profile, it can be seen that the profile increased sharply as the extraction began, and later decreased indicating that the PCBs were extracted from the matrix. A fifteen minutes extraction time was found sufficient to extract the PCBs from the spiked sample. As for the extraction of real samples, a longer time is needed to force the analytes out from the matrix site and the time of extraction was doubled to 30 minutes.

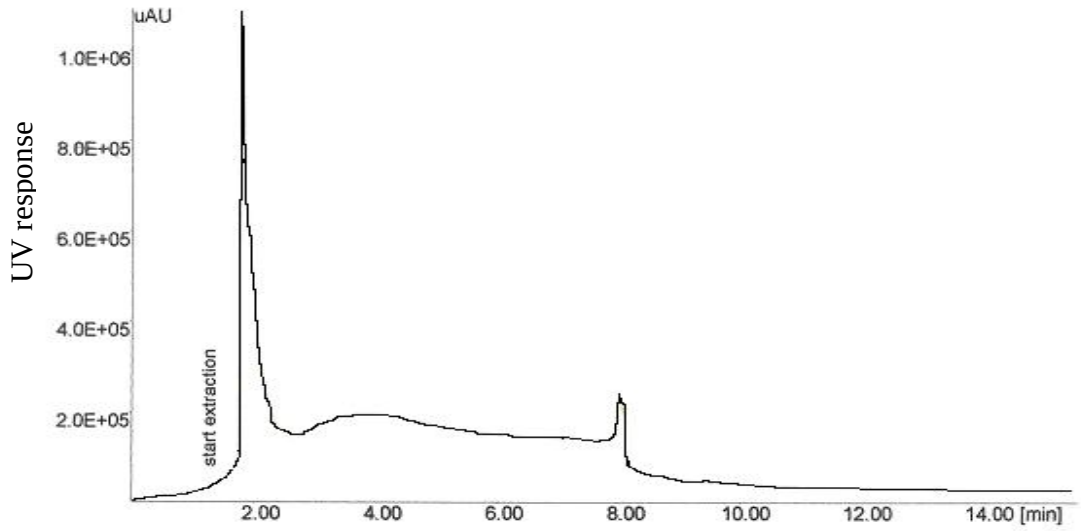


Figure 4.10 Extraction profile for PCBs using a temperature of 60 ^{o}C, pressure of 200 bar and 15 % methanol as modifier.

Therefore, subsequent SFE analyses on real samples were carried out using a 30 minutes extraction time for quantitative recoveries of PCBs (Table 4.4).

Table 4.4 Recoveries of PCB using SFE at optimized condition

PCB No	Percent Recovery ± RSD
28	55.2 ± 2.5
52	91.9 ± 9.2
101	91.0 ± 6.2
118	99.2 ± 1.6
138	85.1 ± 4.6
153	91.4 ± 5.8
180	96.3 ± 3.8

Optimized SFE condition: Pressure = 200 bar, Temperature = 60 ^{o}C,
Modifier = 15 % Methanol, SF flow rate = 2.0 mL/min and extraction time of 30 min

4.7 Subcritical Water Extraction

In this study, an alternative technique using subcritical water was also applied for PCBs extraction. Water is an "environmental-friendly solvent" and has the additional advantages of being readily available, non-toxic, and cheap. Subcritical water extraction was performed using the lab-made SWE extractor as described in Section 2.2.2. For preliminary studies, glass wool was chosen as the matrix for optimization of SWE parameter of PCBs. Glass wool was used to represent a matrix where negligible analyte-matrix interaction is expected. The following parameters were investigated for optimizing the analytes recoveries (i) extraction temperature, (ii) water flow rate, (iii) solvent trapping and (iv) extraction time.

4.7.1 Influence of Extraction Temperature

Temperature is the most important extraction parameter of PCBs. The spiked samples (glass wool) were prepared in triplicates and were extracted at 50 bar for 1 minute in a static condition followed by a 10 minute dynamic extraction. The flow rate was initially set at 1.0 mL/min and the extraction was conducted at different temperatures (50, 100, 150, 200 and 250 ^{o}C).

Figure 4.11 shows the chromatogram for SWE extracts at different extraction temperature. No PCBs were extracted at temperature 50 and 100 ^{o}C. When the water temperature was increased to 150 ^{o}C, the PCBs at low quantities were obtained. Further increased in temperature to 200 and 250 ^{o}C gave increased extraction of all PCBs.

The temperature effect observed during the extraction of PCBs was mainly resulted by the decrease in the polarity of water at higher temperatures (Yang *et al.*, 1995). Raising the water temperature dramatically reduces the dielectric constant of water. Therefore, water behaves like an organic solvent at elevated temperatures.

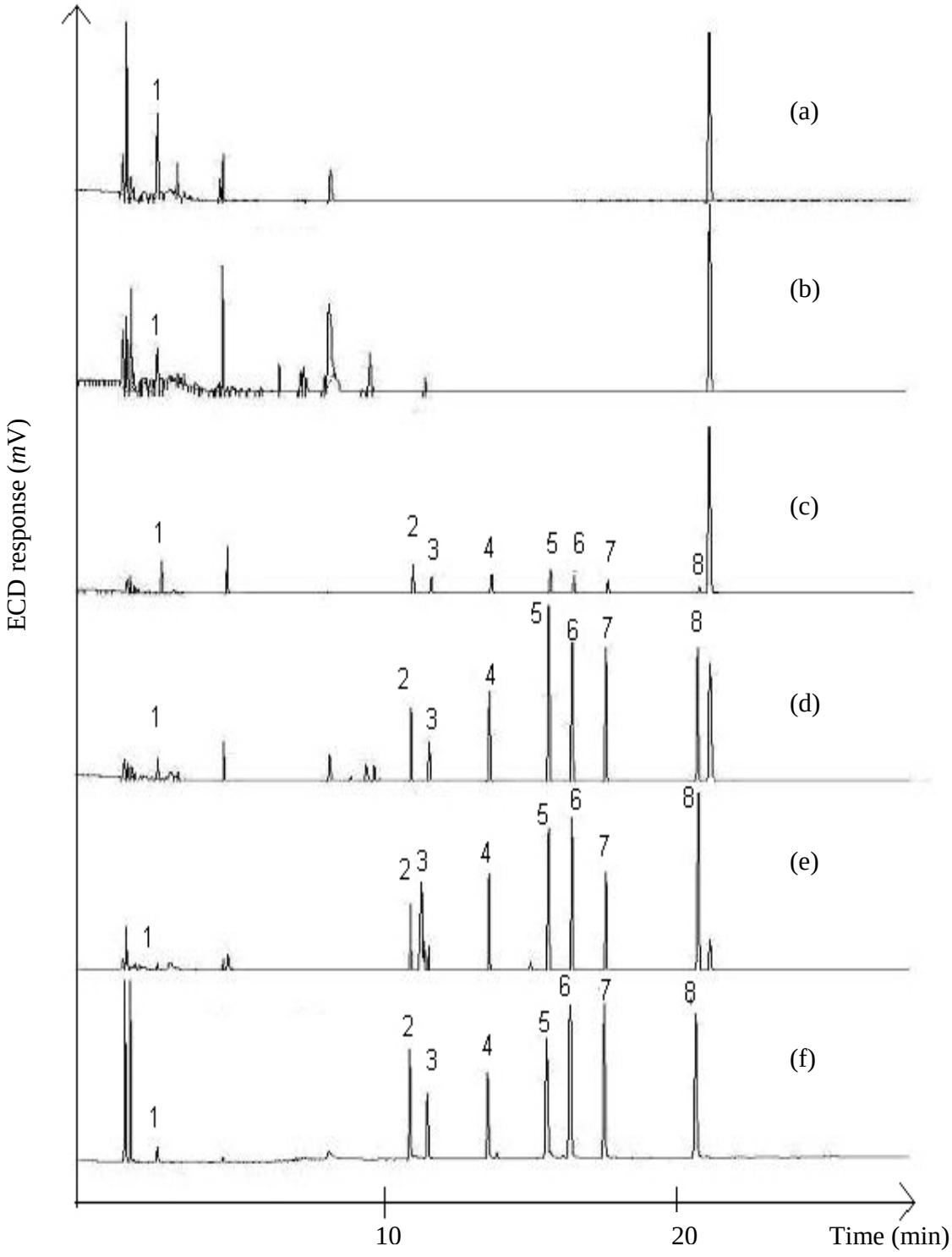


Figure 4.11 GC-ECD Chromatograms for SWE extracts at different extraction temperature (a) 50 °C, (b) 100 °C, (c) 150 °C, (d) 200 °C, (e) 250 °C and (f) PCB standard. Peak identity 1: *iso*-octane 2: PCB 28, 3: PCB 52, 4: PCB 101, 5: PCB 118, 6: PCB 153, 7: PCB 138 and 8: PCB 180. GC conditions as in Figure 4.1.

The low polarity of water at higher temperatures makes it possible to dissolve more organic species so that the solubility of PCB in matrix sample is enhanced during the extraction. Since the extraction efficiency is highly dependent upon the solubility of PCBs in the extraction fluid, a better PCB recovery is yielded at higher temperatures then the one which is extracted using the ambient water. Pressure has little effect on the solvent strength of subcritical water as long as the liquid state is maintained (Yang *et al.*, 1995).

As shown in Table 4.5, no PCBs were extracted at 50 and 100 ^{o}C, whereas at 150 ^{o}C, low recoveries (7 to 27 %) of PCB congeners were obtained. However, as the temperature was increased to 200 ^{o}C, the recoveries of most PCB congener were generally greater than 60 % except PCB 28. Further increment in temperature to 250 ^{o}C yielded better recoveries between 85 to 91 % except for PCB 28 (58 %) and PCB 52 (66 %).

Table 4.5 PCBs removal efficiencies from spiked glass wool at varying extraction temperatures.

Temperature (^{o}C) / PCB	(%)Recovery (RSD*)				
	50	100	150	200	250
28	0	0	15.12 (5.11)	41.24 (5.44)	58.24 (10.02)
52	0	0	16.23 (4.16)	65.65 (4.12)	66.43 (13.21)
101	0	0	27.17 (7.14)	70.22 (10.04)	90.11 (12.32)
118	0	0	20.31 (5.23)	72.16 (15.24)	85.13 (11.20)
138	0	0	10.42 (4.32)	63.23 (9.24)	91.16 (9.24)
153	0	0	15.46 (3.22)	79.26 (13.30)	89.20 (5.12)
180	0	0	7.62 (3.42)	65.17 (12.22)	86.02 (12.10)

*n=3

The result is in good agreement to that obtained by other previous works (Yang *et al.*, 1995; Hartonen *et al.*, 1997 ; Pross *et al.*, 2000) who utilized the temperature at 250 ^{o}C to extract PCBs. The reproducibility of extraction temperature on SWE was shown to be comparatively good with all samples having RSD below 15.24 %. Hence a temperature of 250 ^{o}C was taken to be the optimal extraction temperature for SWE.

4.7.2 Influence of Water Flow Rate

For dynamic SWE, different flow rates of extractant were tested in an attempt to improve the extraction efficiency. Triplicate extractions were performed at 250 ^{o}C and 50 bar using 0.5, 1.0, 1.5 and 2.0 mL/min. The condition applied for each extraction was 1 minute static extraction followed by 10 minutes dynamic extraction. The percentage recoveries of PCBs congener obtained using different flow rates are depicted in Figure 4.12. At a flow rate of 0.5 mL/min, PCB congeners were extracted in the range of in 45 to 77 %. However, as the water flow rate was increased to 1.0 mL/min, the extractions of all PCB congener were higher compared to the one with flow rate of 0.5 mL/min. When the flow rate was increased to 1.5 mL/min, the percentage recoveries of PCB were slightly increased. Further increment up to 2.0 mL/min gave reduced recoveries of PCB congeners.

A higher flow rate produced a large amount of extract solution which was collected in the sample vial. Hence, the concentration of all PCB in the water extract was too low posing difficulty for organic solvent removal from the water extract. Therefore the optimum flow rate chosen in this study was 1.0 mL/min. The reproducibility of extraction flow rate on SWE was shown to be satisfactory with all samples displaying RSD below 12.1 %.

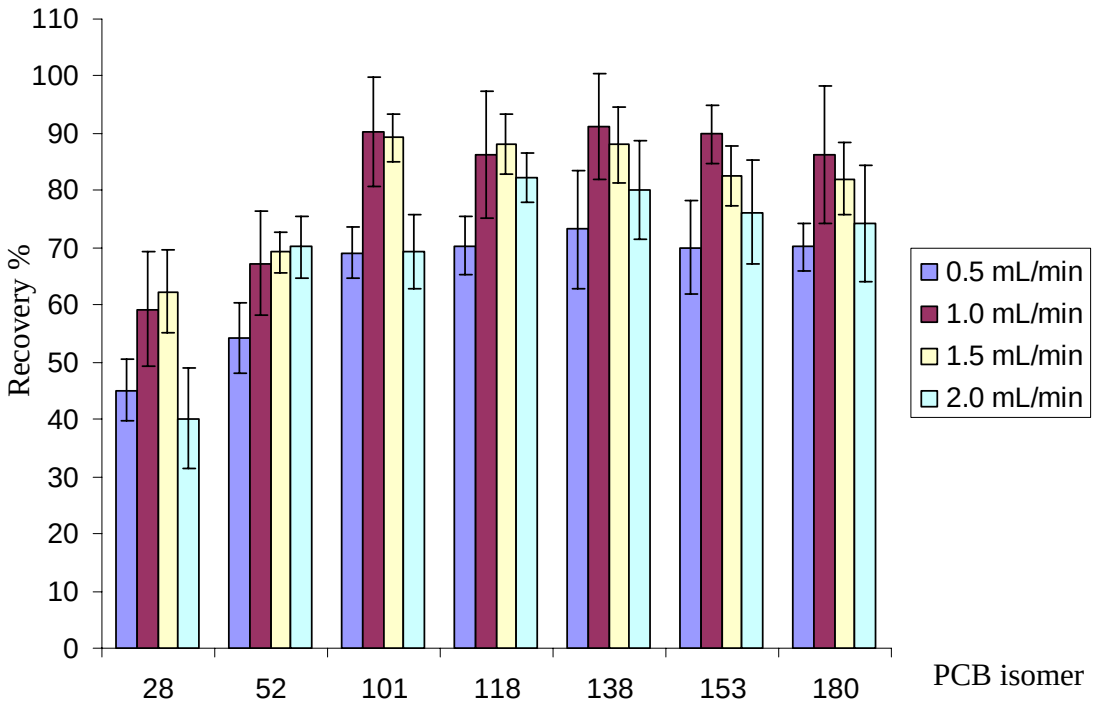


Figure 4.12 Effect of extraction water flow rate on the extraction efficiency of all PCBs congener from spiked glass wool using SWE at 250 ^{o}C and 50 bar, 1 minute static, 10 minute dynamic extraction and collect in 5 mL DCM.

4.7.3 Effect of Trapping Solvent

The use of particular solvent to collect and trap the extract greatly influences the extraction sensitivity. Dichloromethane, *n*-hexane, *iso*-octane and *n*-heptane were tested as possible analyte trap solvents. Reasonably good collection efficiencies with dichloromethane (58 % to 91 %) were obtained for individual PCB congeners (Figure 4.13). Unfortunately, none of the non-halogenated solvents could efficiently collect the PCBs from the water extracts. The trapping efficiencies were typically 30 to 70 % for *n*-heptane, 35 to 66 % for *n*-hexane and 23 to 67 % for *iso*-octane.

Although Hartonen *et al.*, (1997) reported that *n*-heptane has good solvent trapping capability PCBs with recoveries in the range 92 to 97 %. The result in work

gave unsatisfactory recovery (26 % to 69 %) using *n*-heptane as the analyte trap solvent. The reproducibility of trapping solvent on SWE was shown to be comparatively good with all samples having RSD below 14.7 %.

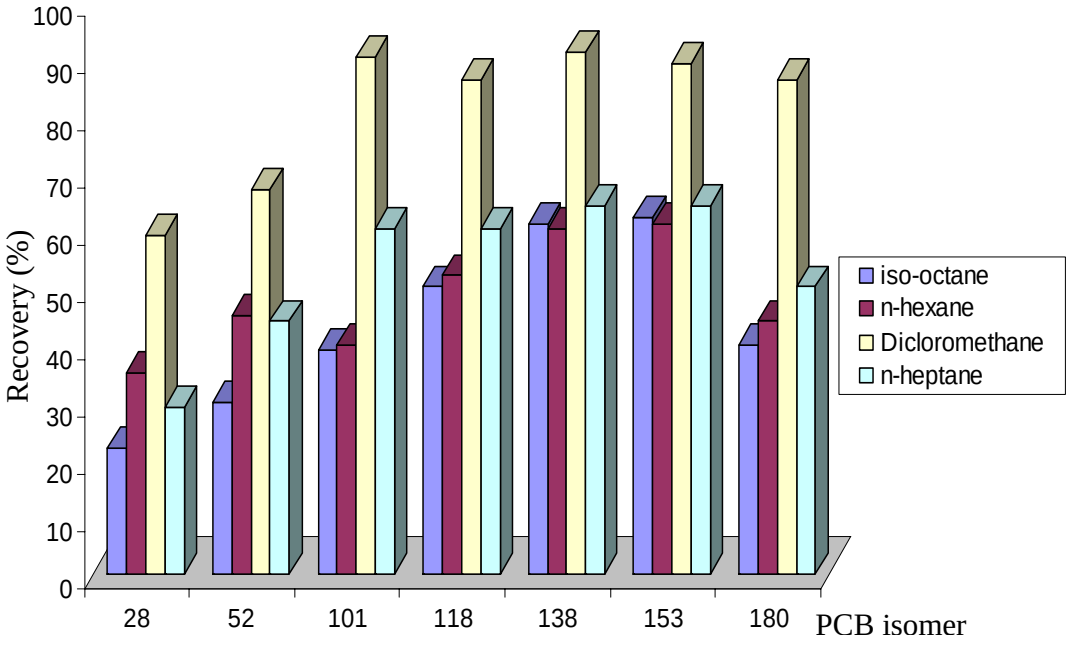


Figure 4.13 Effect of solvent trapping on the extraction efficiency of all PCBs congener from spiked glass wool using SWE at 250 ^{o}C and 50 bar, 1 minute static, 10 minute dynamic extraction and collect in 5 mL DCM.

4.7.4 Effect of Duration Time

Extraction time is the one of the important extraction parameters of PCBs that gives effect to the efficiency extraction. As shown in Figure 4.14, an extraction time of 5 minutes could only extract 30 to 65 % of the PCB congeners. However the recoveries increased to 55 to 91 % when the duration was increased to 10 minutes. At longer extraction time of 15 and 20 minutes, percentage recoveries were found to decrease slightly.

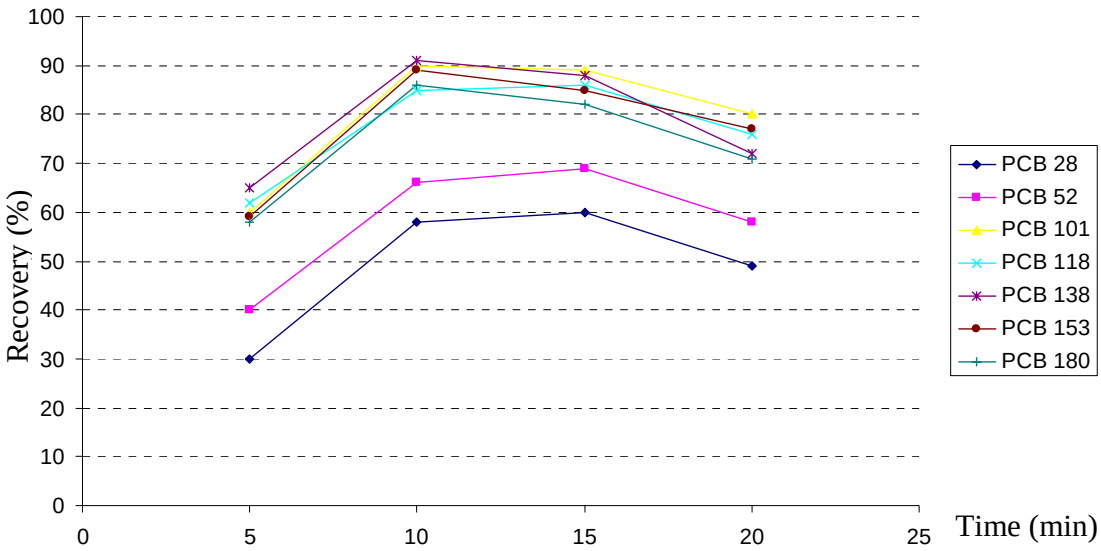


Figure 4.14 Effect of extraction time on the extraction efficiency of PCBs congeners from spiked glass wool using SWE at 250 ^{o}C and 50 bar, 1 minute static, 10 minute dynamic extraction and collect in 5 mL DCM.

Yang *et al.*, (1995) reported a 15 minutes extraction for the optimal extraction time of PCB. If the water extraction was performed for 20 minutes, approximately 20 mL of water extract will be collected using an extractant flow rate 1.0 mL/min. At the longer extraction time, the higher volume of water extract would therefore be collected. This would cause an apparent low concentration of extracted PCBs in extract. As a result, 10 minutes extraction time was chosen as the suitable period for the subsequent SWE.

4.7.5 PCB Recoveries from Spiked Sewage Sludge

The optimized SWE conditions of PCBs for spiked glass wool were applied to the extraction of PCBs for spiked sewage sludge. The GC chromatogram for SWE

extract for spiked sludge samples showed several spurious artifacts peaks that occur between 6 to 13 minute (Figure 4.15). These peaks could be due to the presence of sulfur in the sludge matrix. To overcome the problem, the SWE extract was subjected to further clean up using activated copper for the removal of sulfur (Bowadt, and Johansson, 1994). As shown in Figure 4.15c, the chromatogram was cleaner and with less interfering peaks.

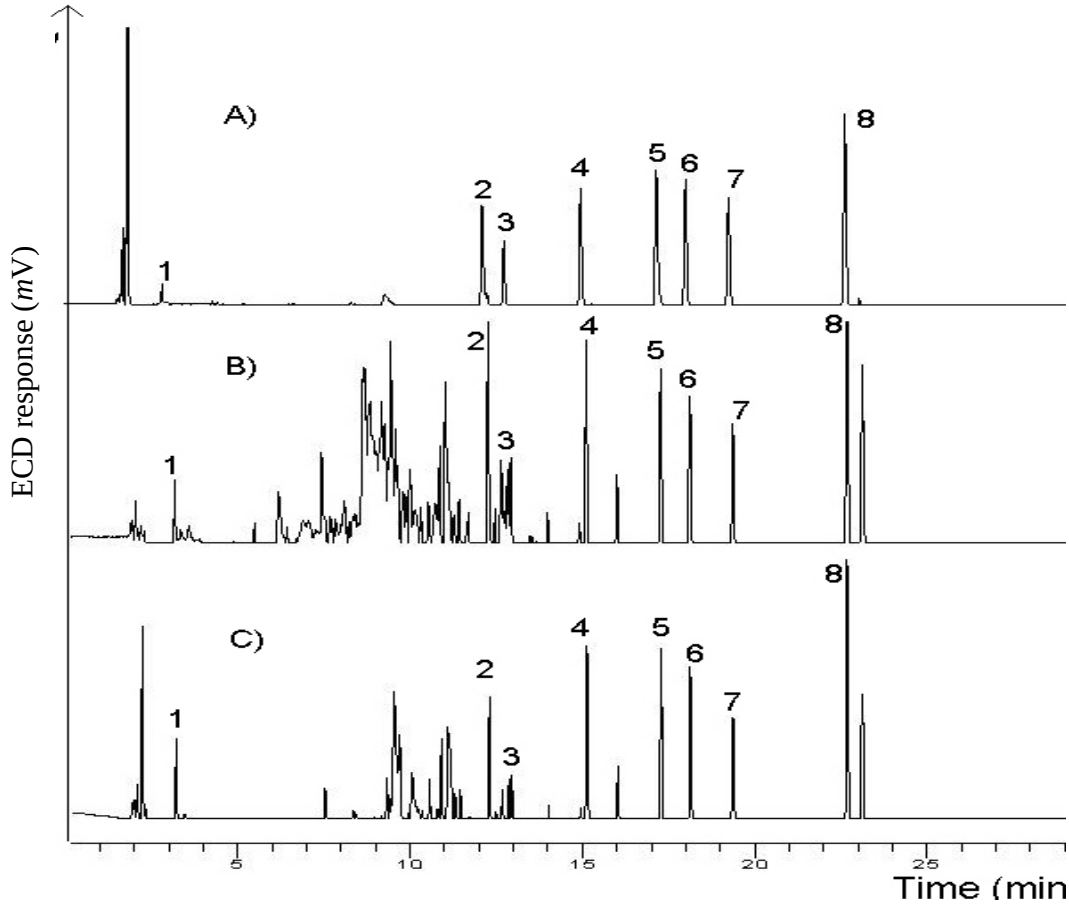


Figure 4.15 The chromatogram GC-ECD for SWE. A) Standard mixture PCB, B) Before treatment with Cu and C) After treatment with Cu. Peak identity 1: *iso*-octane 2: PCB 28, 3: PCB 52, 4: PCB 101, 5: PCB 118, 6: PCB 153, 7: PCB 138 and 8: PCB 180.

The results (Figure 4.16) showed that, PCBs for the spiked glass wool were easily extracted and gave better recoveries then those from the spiked sewage sludge. This may be due to the difference in the nature sample of a matrix. The recovery of all PCBs spiked sludge sample ranged from 50 to 80 %. The lower PCBs displayed recoveries in range of 50 to 60 %. For both the spiked sample, all RSD were lower than 10 % indicating satisfactory reproducibility of SWE technique.

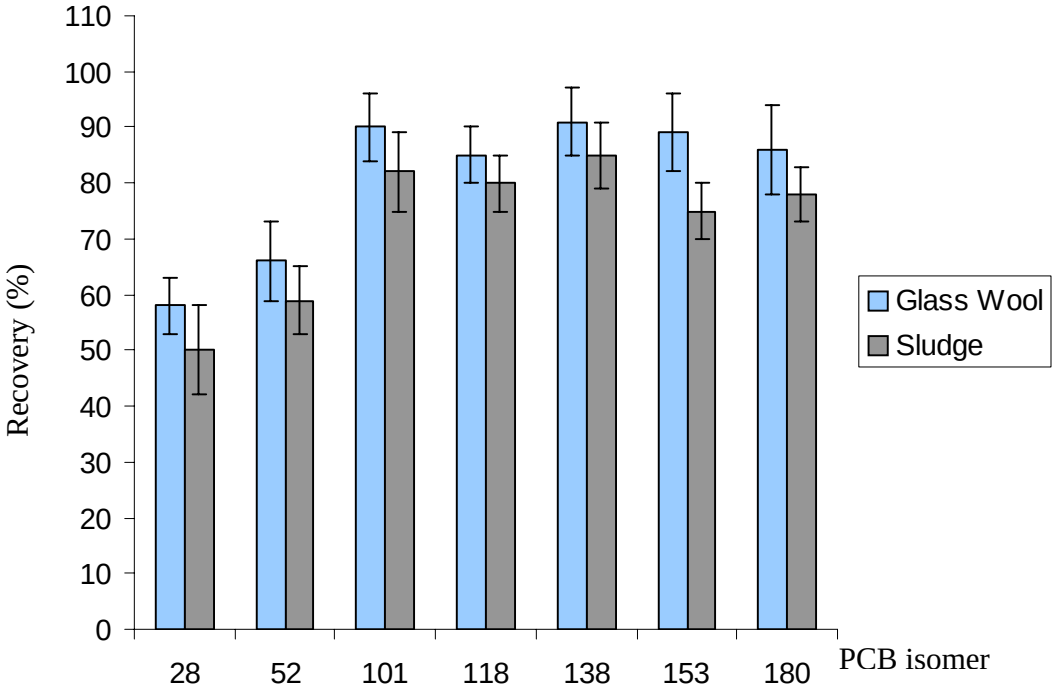


Figure 4.16 Effect of different sample matrix on the SWE recoveries PCBs Optimum SWE conditions: temperature = 250 ^{o}C, pressure = 50 bar, flow rate = 1.0 mL/min, 1 minute static, 10 minute dynamic extraction and collect in 5 mL DCM.

4.6 Comparison of Extraction Techniques

Table 4.6 lists the comparison of the three techniques applied for the extraction of PCBs. The organic Soxhlet extracts were much darker and highly turbid (in dark yellow), while the extracts from subcritical water (collected in dichloromethane) were

orange and somewhat turbid, and the extracts from SFE (collected in *n*-hexane) were pale yellow and also a little turbid. The organic soxhlet extracts also yielded more artifact peaks in the GC–ECD chromatograms, especially compared to supercritical CO_2 and subcritical water.

Table 4.6 Comparison of extraction techniques.

	Soxhlet	SFE	SWE
Extraction Solvent	Dichloromethane-Acetone	CO_2 + Methanol modifier	Water
Collection Solvent	-	*n*-hexane	Dichloromethane
Volume of solvent use	Large (300 mL)	Small (30 mL)	Small (15 mL)
Pressure (bar)	Ambient	200	50
Temperature (^{o}C)	~40-60	60	250
Extraction time	24 hour	30 minute	15 minute
Color of extract	Dark yellow	Pale yellow	Light orange
Extract clean-up	Required	Not required	Not required

Large volumes of solvent are necessary for traditional soxhlet extraction compared to SFE and SWE method. The need to dispose of these solvents presents the potential for further environmental contamination and expense. Soxhlet extraction is also very time consuming compare than SFE and SWE method only needed 30 minute to extract analyte for the sample.

The PCB recovery to which all other result was based on soxhlet extraction for 24 hour using n-hexane/acetone (1:1, v/v) is shown in Table 4.7. *n*-hexane is too non polar and dichloromethane is halogenarated. In this study, *n*-hexane/acetone was applied as extraction solvent based on previously research. From the table 4.7, both the new extraction techniques SFE and SWE show good recoveries from 50 to 99 % compared with soxhlet extraction. SFE and SWE the added advantages of low organic solvent

usage, short extraction and environmentally clean technique compared conventional technique. The result is in good agreement to that obtained by other previous works by Sporring *et al.,* (2000) and Mannila *et al.,* (2002) PCBs obtained by SFE corresponded well with those obtained by soxhlet method. SWE and SFE have the most ability to change solvent conditions by controlling simple parameters (temperature and pressure for SFE and primarily temperature for SWE) may have the most potential for extracting mobile versus bound pollutant molecules from solid environmental.

Table 4.7 Comparison of PCBs recovery using Soxhlet, SFE and SWE

Extraction / PCB No	Percent Recovery ± RSD		
	Soxhlet	SFE	SWE
28	52.2 ± 4.2	55.2 ± 2.5	50.3 ± 8.2
52	58.2 ± 3.2	91.9 ± 9.2	59.1 ± 6.3
101	74.1 ± 5.0	91.0 ± 6.2	82.5 ± 7.1
118	70.8 ± 2.2	99.2 ± 1.6	80.2 ± 5.9
138	72.3 ± 3.2	85.1 ± 4.6	85.1 ± 6.2
153	74.4 ± 2.6	91.4 ± 5.8	75.3 ± 5.1
180	75.2 ± 4.2	96.3 ± 3.8	78.1 ± 5.3

4.5 Summary

All seven PCBs congener were successfully separated, with a total time less than 30 minutes using GC-ECD with good linearity and low detection limit. SFE has been shown to be effective for the PCB extraction method or removal from the sludge matrix. The optimized SFE condition used extraction temperature of 60 ^{o}C, extraction pressure of 200 bar, 15 % methanol as co solvent, extraction flow rate at 2.0 mL/min, 30 minutes extraction and *n*-hexane as the trapping solvent. For the SWE, the best condition for the

extraction of all PCBs were at a temperature of 250 ^{o}C and pressure of 50 bar, 1 min static followed by 10 min dynamic extraction and analyte collection in dichloromethane.

Both the new extraction techniques of SFE and SWE showed good PCB recoveries ranging from 50 to 99 % compared to soxhlet extraction. SFE and SWE gave added advantages of low organic solvent usage, short extraction time and environmentally clean technique. From the two extraction methods investigated, it was clear that the SFE technique was better in terms of recovery, good reproducibility compared to SWE method and it was therefore chosen as the method to be used for the analysis of real sludge samples.

CHAPTER 5

ANALYSIS OF PCBs IN REAL SAMPLES

5.1 Introduction

The analysis of PCBs level in real sewage sludge samples will be discussed in this chapter. Extraction was conducted according to the optimization SFE parameters (refer Chapter 4), employing an extraction temperature of 60 ^{o}C, extraction pressure of 200 bar, 15 % methanol as co solvent, extraction flow rate at 2.0 mL/min, 30 minutes extraction and *n*-hexane as the trapping solvent. Samples were first analyzed with GC-ECD, and peak confirmations were performed with co-injection and GC-MS techniques. SFE method was then compared with Soxhlet extraction.

5.2 Level of PCBs in Sludge Sample

Figure 5.1 showed the GC-ECD chromatograms of sludge samples extracted using SFE method. The results showed that PCB isomers were only detected in four sludge samples. The sludge samples that contained the isomers of PCBs were TI, TY, LK and KIS.

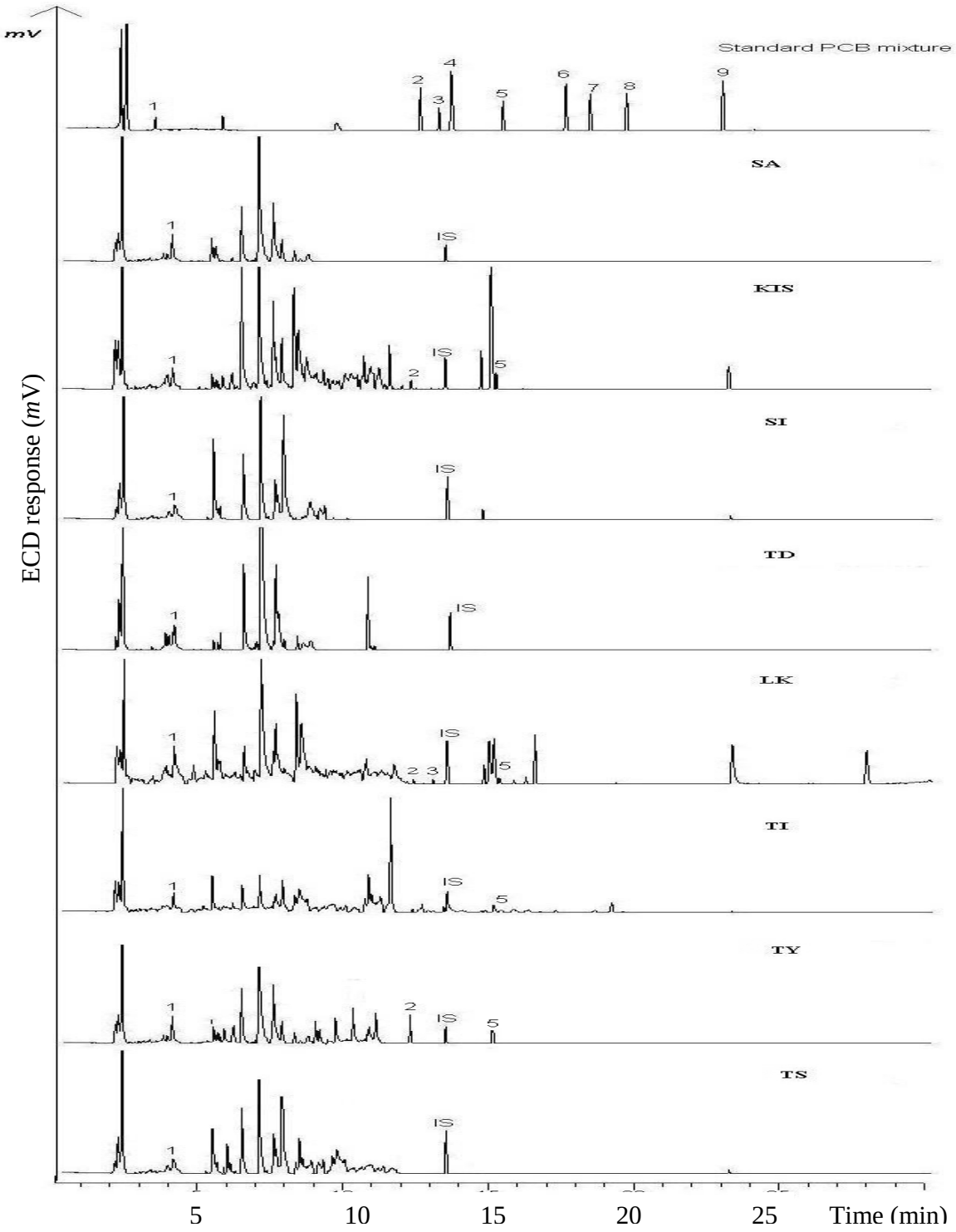


Figure 5.1 GC-ECD chromatograms for SFE extracts real sample sludges. Peak identity: (1) *iso*-octane (2) PCB 28, (3) PCB 52, (4) PCB 35, (5) PCB 101, (6) PCB 118, (7) PCB 153, (8) PCB 138, and (9) PCB 180. (IS) internal standard PCB 35.

TI sludge samples contained PCB 101 only, while both samples TY and KIS were found to contain PCB 28 and 101. PCB 28, 52 and 101 were found in LK sludge samples, no PCBs were detected in other sludge samples. Table 5.1 showed the concentration of PCBs in real sample sludge extracted using the SFE method. As shown in Table 5.1, PCBs were found in low concentration in the range 31.2 to 82.0 µg/kg. Sludge sample from TY showed slightly higher concentration of PCB 28 and PCB 101 with 75.4 µg/kg and 82.0 µg/kg respectively. This higher level of PCB in TY sample could be attributed to the electronic industry situated in front of this wastewater treatment plant. Sludge samples from LK, KIS and TI also contained PCBs due to the fact that situated the WTP are light industrial area arising from industries such as battery, electronic, metal and plastic manufacturing.

Table 5.1 PCB levels in sludge sample using SFE

PCB / sample	Concentration (µg/kg) ±RSD						
	28	52	101	118	138	153	180
SA	ND	ND	ND	ND	ND	ND	ND
TD	ND	ND	ND	ND	ND	ND	ND
TI	ND	ND	31.2(1.9)	ND	ND	ND	ND
LK	50.1(1.4)	30.6(2.1)	51.3(3.2)	ND	ND	ND	ND
KIS	69.3(3.5)	ND	60.1(5.2)	ND	ND	ND	ND
TY	75.4(3.8)	ND	82.0(5.5)	ND	ND	ND	ND
TS	ND	ND	ND	ND	ND	ND	ND

ND= Not detected, n=3

Table 5.2 showed the concentration of PCBs in real sample sludge extracted using soxhlet extraction. From this table, concentrations of PCB using soxhlet extract were found in the range 20.2 to 60.1 µg/kg. Sludge sample from KIS showed the higher

level of PCB especially PCB 101 with 60.1 µg/kg and the lower level of PCB were found from LK sample with 20.2 µg/kg for PCB 101. According to the results reported by Abad *et al.,* (2005) low concentrations of PCBs in sludge in the range from 3 to 60 µg/kg were obtained far below the European recommended limit for PCB. Similarly, Blanchard *et al.,* (2004) found PCB concentrations from 70 to 650 µg/kg dm in sludge samples taken in a WTP which drained the Paris area. Work by Paulsrud *et al.,* (1998) reported PCB concentrations ranging from 17 to 100 µg/kg dm, with a median value of 42 µg/kg in Norwegian sludges.

Table 5.2 PCB levels in sludge sample using Soxhlet extraction

PCB / Sample	Concentration (µg/kg) ±RSD						
	28	52	101	118	138	153	180
SA	ND	ND	ND	ND	ND	ND	ND
TD	ND	ND	ND	ND	ND	ND	ND
TI	ND	ND	ND	ND	ND	ND	ND
LK	31.8(2.5)	32.4(3.1)	20.2(5.2)	ND	ND	ND	ND
KIS	40.2(4.2)	ND	32.1(3.4)	ND	ND	ND	ND
TY	51.2(5.6)	ND	60.1(4.5)	ND	ND	ND	ND
TS	ND	ND	ND	ND	ND	ND	ND

ND= Not detected, n=3

In this work, concentration levels of PCB in sludge samples using soxhlet were found lower than using SFE. These results were supported by the work of Mannila *et al.,* (2002), who obtained higher values of PCBs by SF-CO_2 compared to that using soxhlet extraction. In this study, all sludge samples contained PCBs level below the limit standard of PCBs in sludge for other countries.

5.3 Analyte Peak Confirmation

It is questionable whether the identified peaks in the GC chromatograms were actually due to PCB peaks. These peaks could be any other co-extracted contaminant, or possibly just noise peaks. Therefore confirmatory measure was carried out using co-injection and GC-MS techniques.

5.3.1 Co-injection

Co-injection is a technique in which a small volume of the standard sample is injected together with the sample extracts. The peak area of the suspected peak at the same retention time will tend to increase. In this study, co-injection technique was used to confirm the existence of PCBs in the sludge sample. Comparison using retention time of PCBs is not accurate because there might be other contaminants in the sample that happened to have the same retention time as the PCB standard under study.

Figure 5.2 shows that the chromatograms for the sludge samples before and after the PCBs standard mixture was added. From the chromatograms of co-injection the concentration of these seven PCBs were totally increased, the peak area for these seven PCB compounds were significantly increased compared to the chromatogram prior to co-injection.

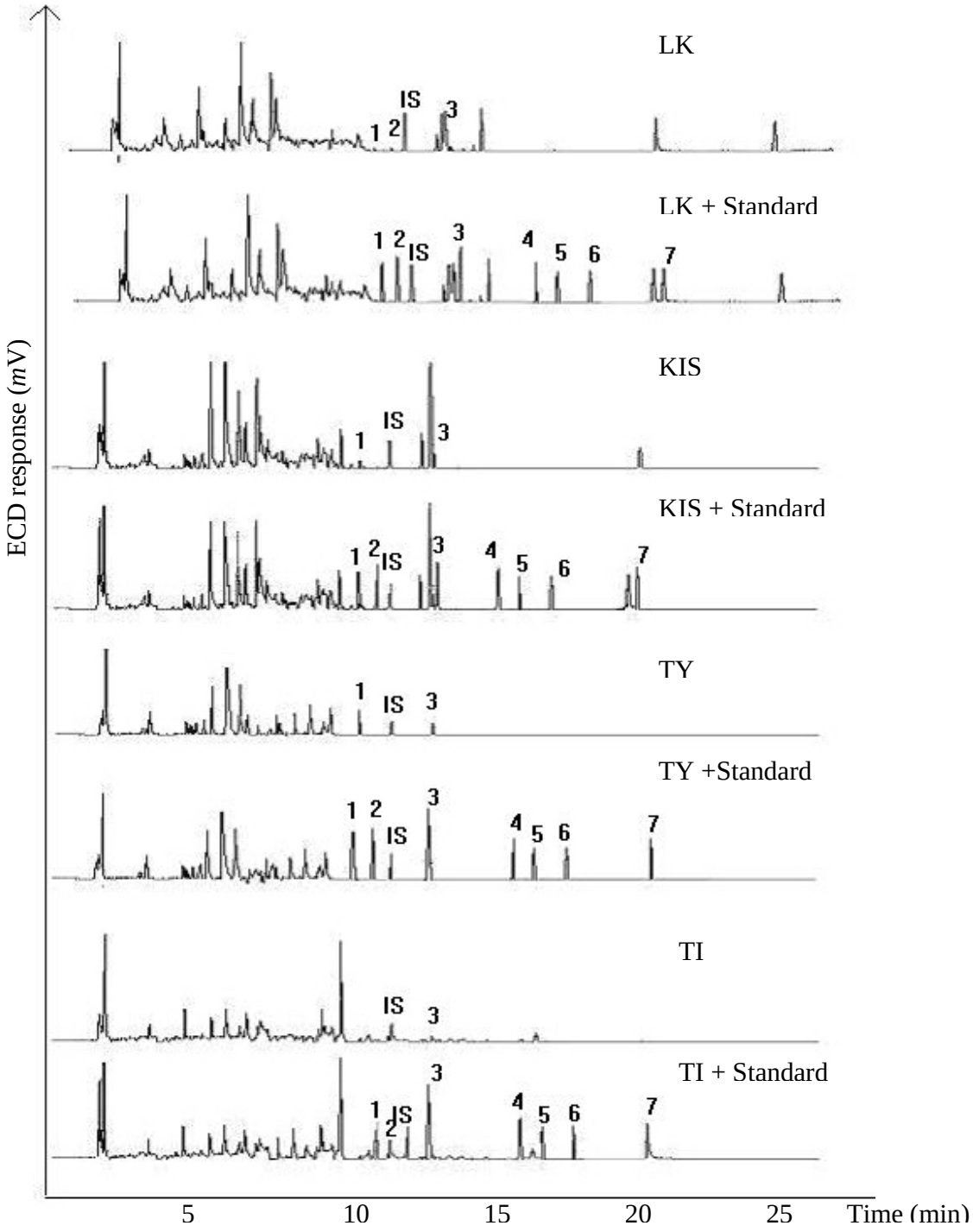


Figure 5.2 The chromatograms for the sludge samples, (LK, KIS, TY and TI) before and after addition of PCB standard mixture. Peak identity: (1) PCB 28, (2) PCB 52, (3) PCB 101, (4) PCB 118, (5) PCB 153, (6) PCB 138, (7) PCB 180 and (IS) internal standard PCB 35.

5.3.2 GC-MS Identification

Gas chromatography-mass spectrometry (GC/MS) combines the fine separating power of GC with the uniquely powerful detection capabilities of MS. This powerful technique is particularly suitable for the analysis of mixtures of volatile and low relative molecular mass compounds such as hydrocarbons, fragrances, essential oils and relatively non-polar drugs.

The MS detector can operate in two different modes; they are scanning and selected ions monitoring (SIM). The scanning mode provides a fairly reproducible mass spectral fragmentation pattern (fingerprint). Mass spectra are recorded (scanned) at regular intervals (typically 0.5 - 1 per second) during the GC separation and stored in the instrument data system for subsequent qualitative or quantitative evaluation. From such patterns, it is often possible to deduce structural features (mass spectral interpretation) but this requires experience and can be very time-consuming, particularly as a complex mixture might contain hundreds of components.

SIM is a much more sensitive technique for trace quantitative analysis. Here, instead of scanning a whole spectrum, only a few ions are detected during the GC separation. Depending on the analyte, low picogram to nanogram amounts can be measured using this powerful technique. Therefore, GC-MS identification using selected ion monitoring utilised for the confirmation of PCBs peak in this study.

The total ion chromatogram for TY sample using selected ion monitoring is shown in Figure 5.3. Figure 5.4 shown the extracted ion chromatograms for TY sample to analyte peak confirmation of PCB 28. The mass for PCB 28 was selected with m/z 150, 256 and 258 (Sulkowski and Rosinska, 1999). The spectrum showed that sample TY contained PCB 28. Figure 5.5 showed the extracted ion chromatograms for PCB 101 with m/z 254, 324 and 326. The spectrum showed the presence of PCB 101 due to the fact that it contained the same m/z for PCB 101. The total ion chromatogram and

extracted ion chromatogram for KIS, LK and TI sample are shown in Appendix C. Appendix D showed the mass spectrum for each PCB isomer.

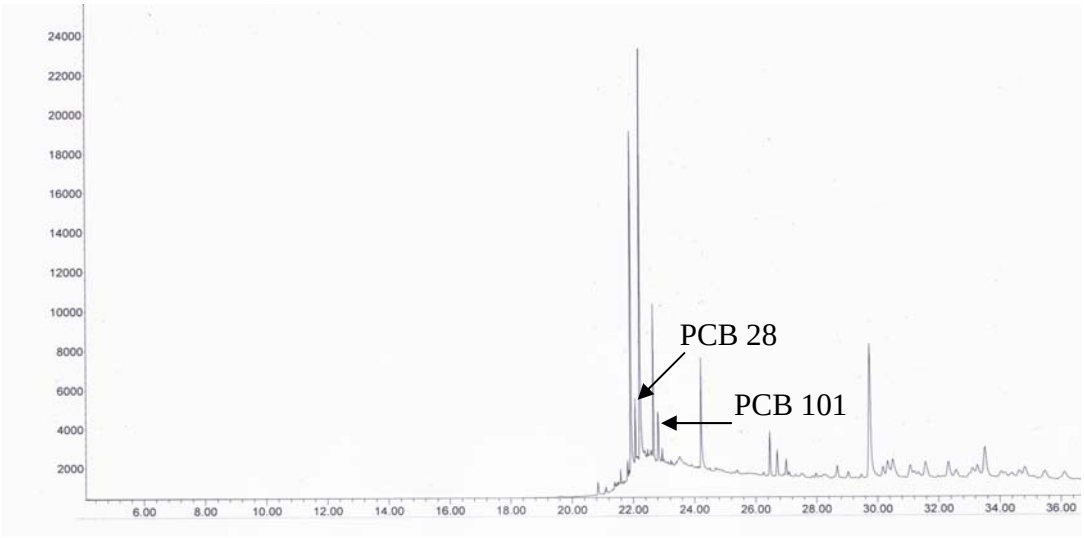


Figure 5.3 Total ion chromatogram of sample TY using GC-MS-SIM mode.

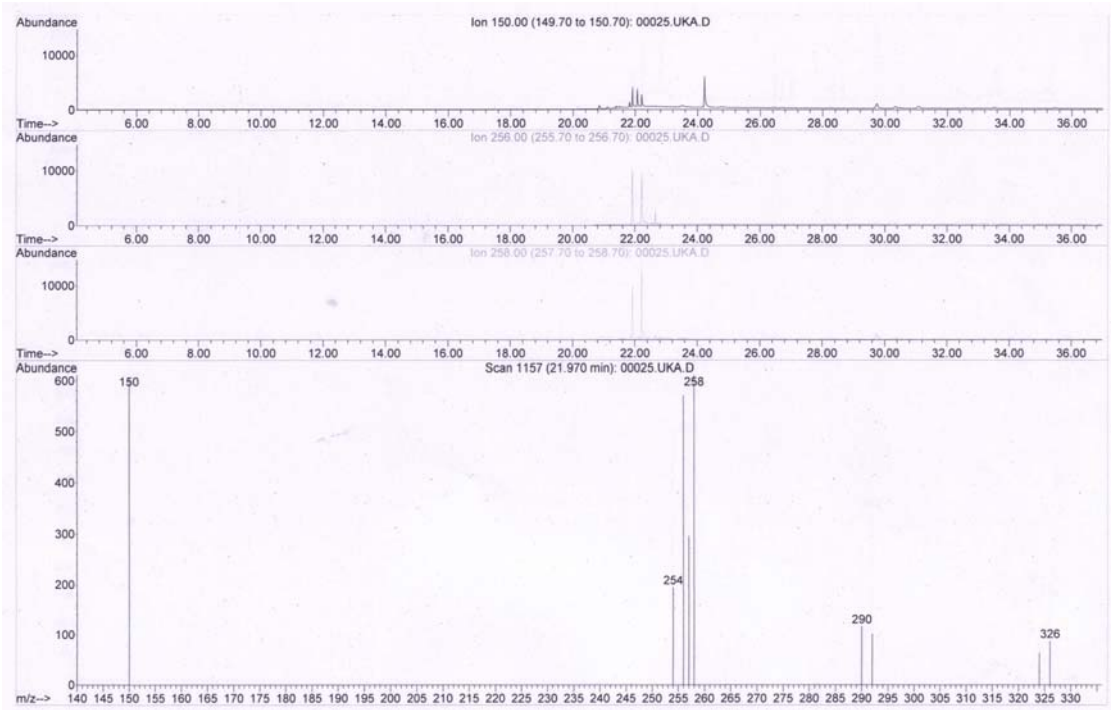


Figure 5.4 Extracted ion chromatogram for sample TY to confirm PCB 28 (m/z 150, 256 and 258).

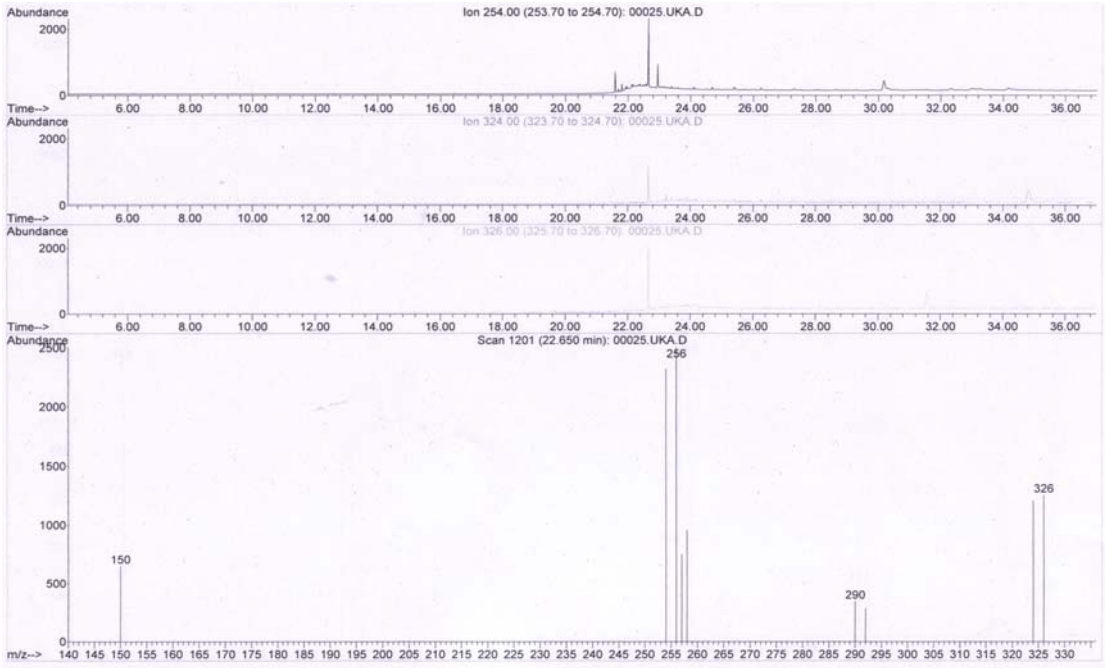


Figure 5.5 Extracted ion chromatogram for sample TY to confirm PCB 101 (m/z 254, 324 and 326).

5.4 Summary

The qualitative and quantitative analysis of PCB in real sewage sludge was carried out in this study using SF-CO_2 and comparison with soxhlet extraction. The concentration of PCBs in sludge extracted using SF-CO_2 was in the range of 31.2 to 82.0 µg/kg. Sludge sample from TY showed slightly higher concentration of PCB 28 and PCB 101 with 75.4 µg/kg and 82.0 µg/kg respectively. Concentrations of PCB using soxhlet extraction were found in the lower range of 20.2 to 60.1 µg/kg. Analyte peak confirmation was carried out using co-injection and GC-MS utilising the SIM mode.

CHAPTER 6

ANALYSIS OF POLYCYLIC AROMATIC HYDROCARBONS

6.1 Separation of the PAH mixture using GC-FID

Separation of the PAH mixture (200 mg/L) was carried out with GC-FID using an Ultra-2 capillary column. Five types of PAH of common occurrence in sewage sludge were chosen. The target analytes were naphthalene, fluoranthene, phenanthrene, benzo(a)fluoroanthene and benzo(a)pyrene. Based on Figure 6.1, All PAHs were successfully separated, with a total time of less than 44 minutes. *n*-alkane C_{16} hydrocarbon was use as an internal standard and eluted between naphthalene and phenanthrene.

6.2 Limits of Detection

In order to determine the sensitivity of the technique, the limit of detection (LOD) of each PAH was determined by the analyte signal three times the background signal. Detection limits for the PAHs are shown in Table 6.1.

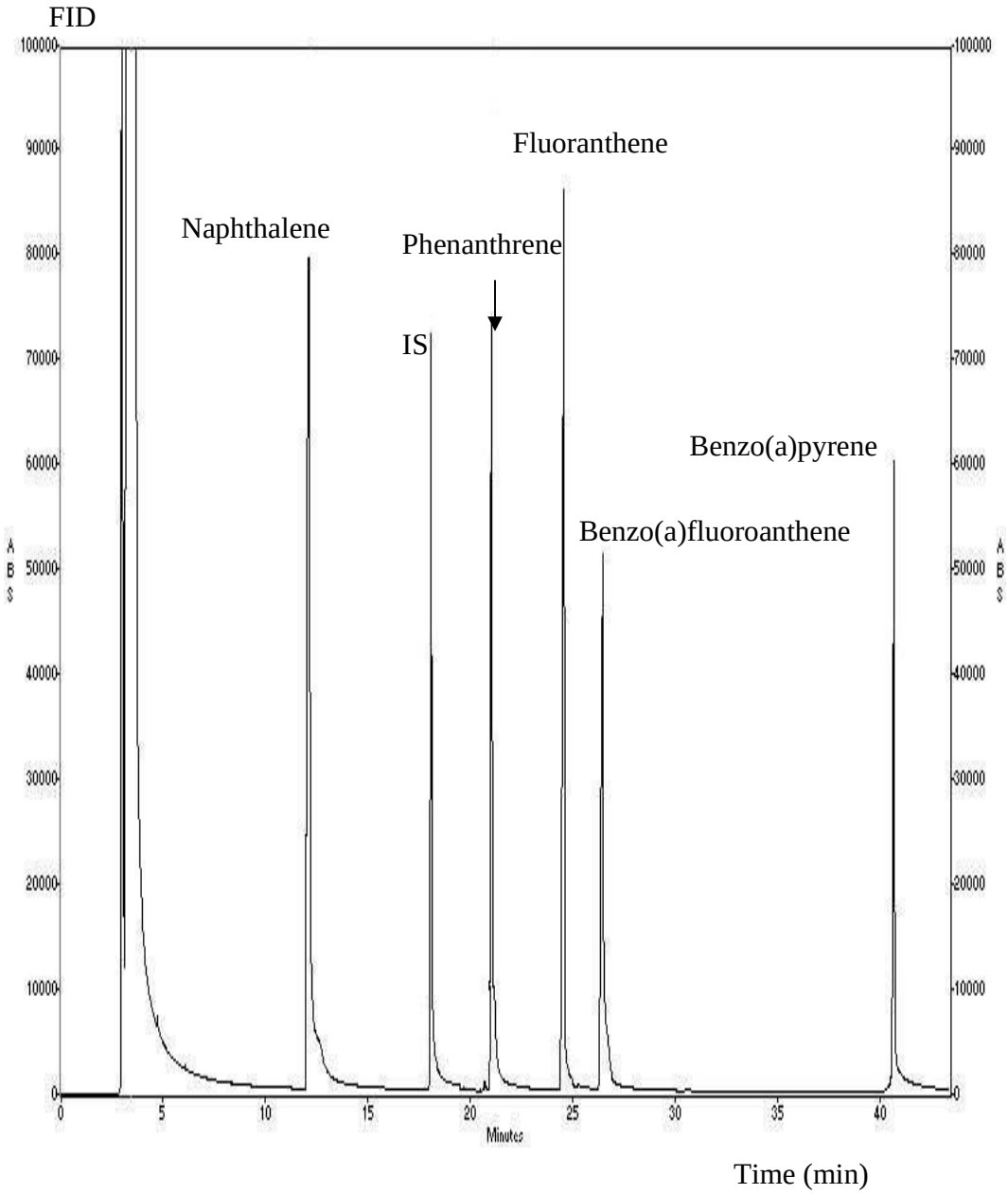


Figure 6.1 GC-FID separation of the PAH using a Ultra-2 capillary column. GC Condition: Injection port temperature at 250 ^{o}C and detector temperature at 300 ^{o}C. Temperature programming from 50 ^{o}C (held for 2 minutes) then ramped to 265 ^{o}C (held for 20 minutes) at a rate of 10 ^{o}C/min.

Table 6.1 Comparison of limit of detection for PAH

	Limit of detection	
PAH	In this study (mg/kg)	[+]Previous study (μg/kg)
Naphthalene	5	40.7
Phenanthrene	10	37.7
Fluoranthene	10	3.0
Benzo(a)fluoranthene	20	-
Benzo(a) pyrene	20	4

[+] Environmental Protection Agency (1986)-Previous study using a GC-FID technique with SE-54 capillary column. GC Condition: Injection port temperature at 280 ^{o}C and detector temperature at 320 ^{o}C. Temperature programming from 35 ^{o}C (held for 2 minutes) then ramped to 265 ^{o}C (held for 12 minutes) at a rate of 10 ^{o}C/min. Flow rate of helium gas at 2 mL/min.

The result showed the lowest limit of detection for naphthalene at 5 mg/kg whereas both of benzo(a)fluoroanthene and benzo(a)pyrene showed high limit of detection with 20 mg/kg. The LOD for all the PAHs in this study were generally higher compared to other study. However, the value of LOD in this study is applicable for analyses of real samples if the quantity of PAH content in sludge is higher than this LOD.

6.3 Calibration Graph of Standard PAH

A calibration graph was constructed to find out the concentration PAH residue in sewage sludge. The resultant sample extract (50 μL) was added with (50 μL) *n*-alkane C_{16} hydrocarbon used as an internal standard (I. S.). Only 1.0 μL of the solution was injected onto the GC. From the calibration graph, the concentration of PAH in sewage

sludge was obtained. The calibration graphs for the five PAHs are shown in Figure 6.2 whereas the linear calibration graph equation and correlation coefficients for five PAH are shown in Table 6.2.

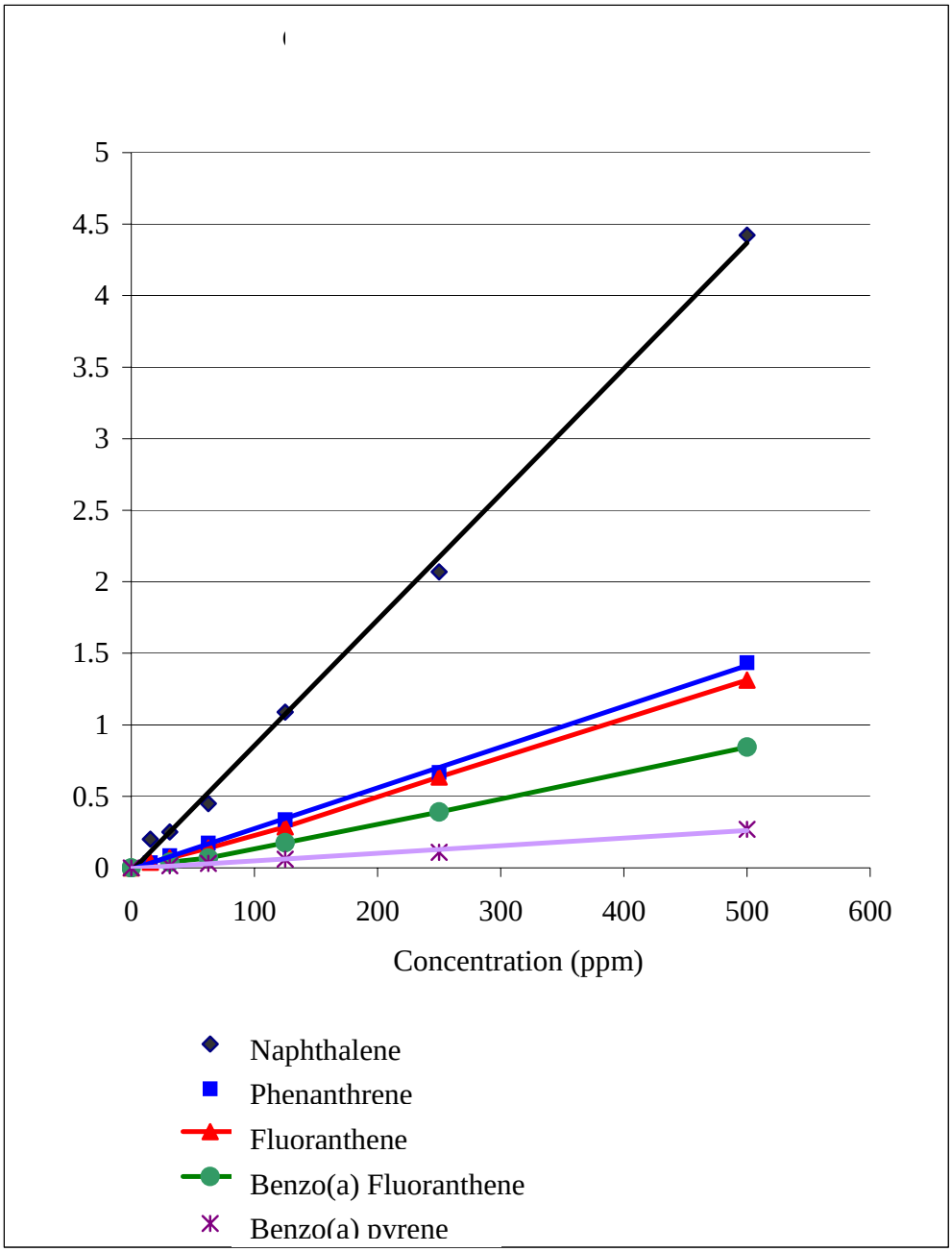


Figure 6.2 Calibration curves for five PAH under study.

Table 6.2 Linear equation and correlation coefficients for each PAH.

PAH	Linear equation	Correlation coefficients
Naphthalene	y = 0.0092x - 0.2007	0.9982
Phenanthrene	y = 0.0029x - 0.0109	0.9988
Fluoranthene	y = 0.0026x - 0.0160	0.9989
Benzo(a)fluoranthene	y = 0.0017x - 0.0231	0.9973
Benzo(a)pyrene	y = 0.0004x + 0.0042	0.9976

The concentration of PAH content in sludge sample can be calculated using the following Equations 6.1

$$\text{PAH (µg/g)} = \text{PAH value from linear equation (µg/mL) x 2 x 1 mL x } \frac{1}{10g} \qquad (6.1)$$

6.4 Evaluation SPE Silica Cartridge with Silica Column

Extraction of PAH for sludge samples were initially conducted using the conventional soxhlet extraction. However, the soxhlet extracts required further extract clean-up prior to GC-FID analyses. Thus, it was desirable to employ an appropriate extract clean-up procedure for PAH sludge analysis. Two most commonly used techniques for sample clean up are the conventional column chromatography and solid phase extraction (SPE) utilising disposable cartridges was chosen for comparison. Table 6.3 showed the comparison of percent recovery of PAH at a spiking level of 200 mg/L.

For overall, the results showed that both procedures gave quantitative recoveries of PAH, however silica gel column was evidently more efficient than the SPE silica cartridge. Based on the GC profile (Figure 6.3), the extract clean-up using silica column

was more efficient than the SPE cartridge due to the less interference peaks in the GC spectrum. Comparison of the SPE cartridge and silica column is as shown in Table 6.4. SPE silica cartridge has some advantages based on reduction of organic solvent consumption, analysis time and reduced mass of silica gel used. However, the ability for adsorption and extract clean-up was less efficient if compared with silica column. The SPE silica was suitable use for less matrix complex and low the concentration PAH. Hence, for this study, the silica gel column was employed for extract clean-up due to less interference peak for GC spectrum and has better ability to absorb PAH.

Table 6.3 Comparison of SPE silica cartridge and silica column

		Silica column	SPE silica cartridge
Volume of solvent	Pentane	90 mL	-
	Hexane	-	25 mL
	CH_2Cl_2	50 mL	-
Time of analysis		120 minute	30 minute
Adsorbed cability		500 mg/L	200 - 300 mg/L
Mass of silica gel		10.0 g	0.5 g
Peak interferences		Less	More

Spiked of volume = 1 mL PAH mixture

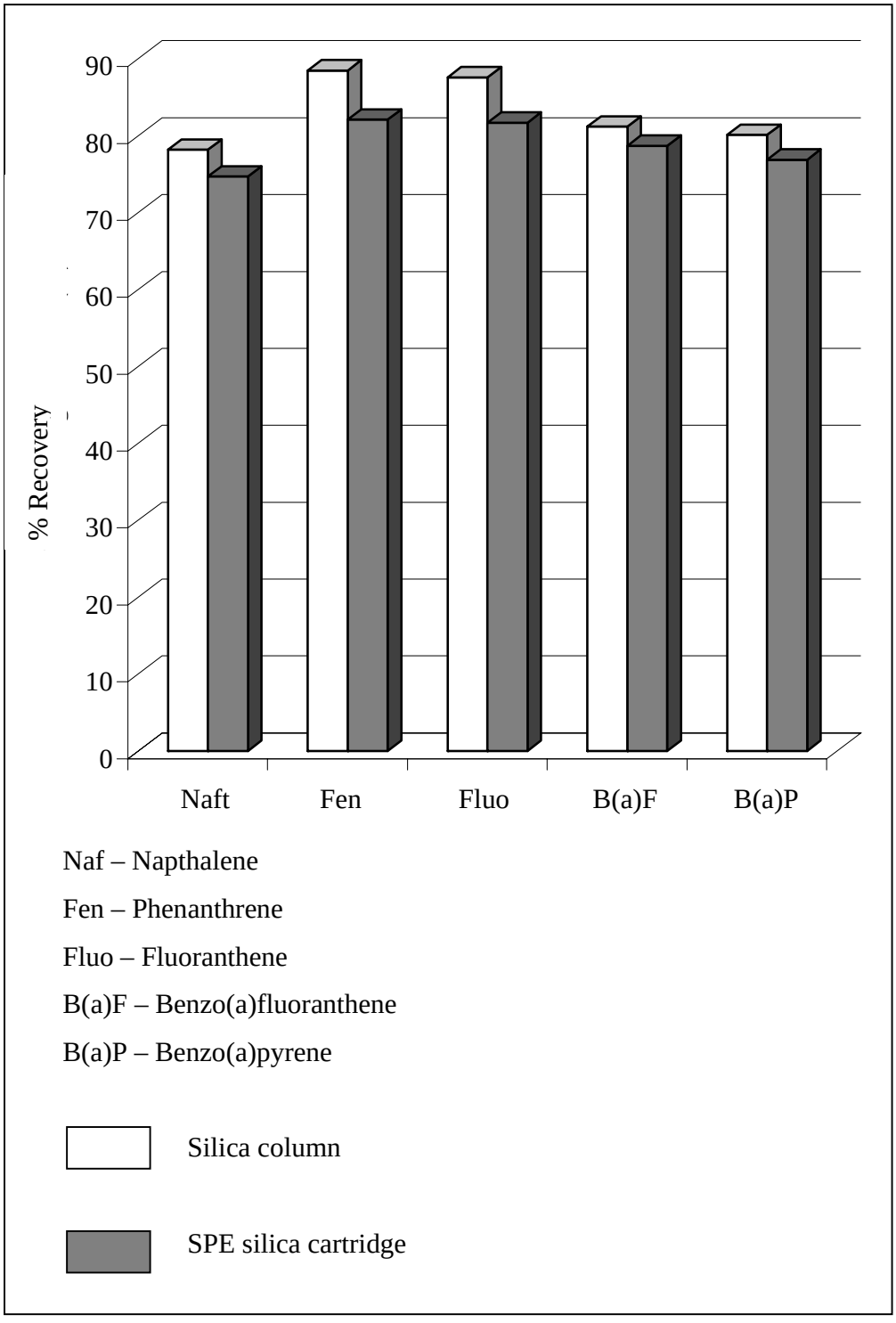


Figure 6.3 PAH recoveries using SPE silica and silica column

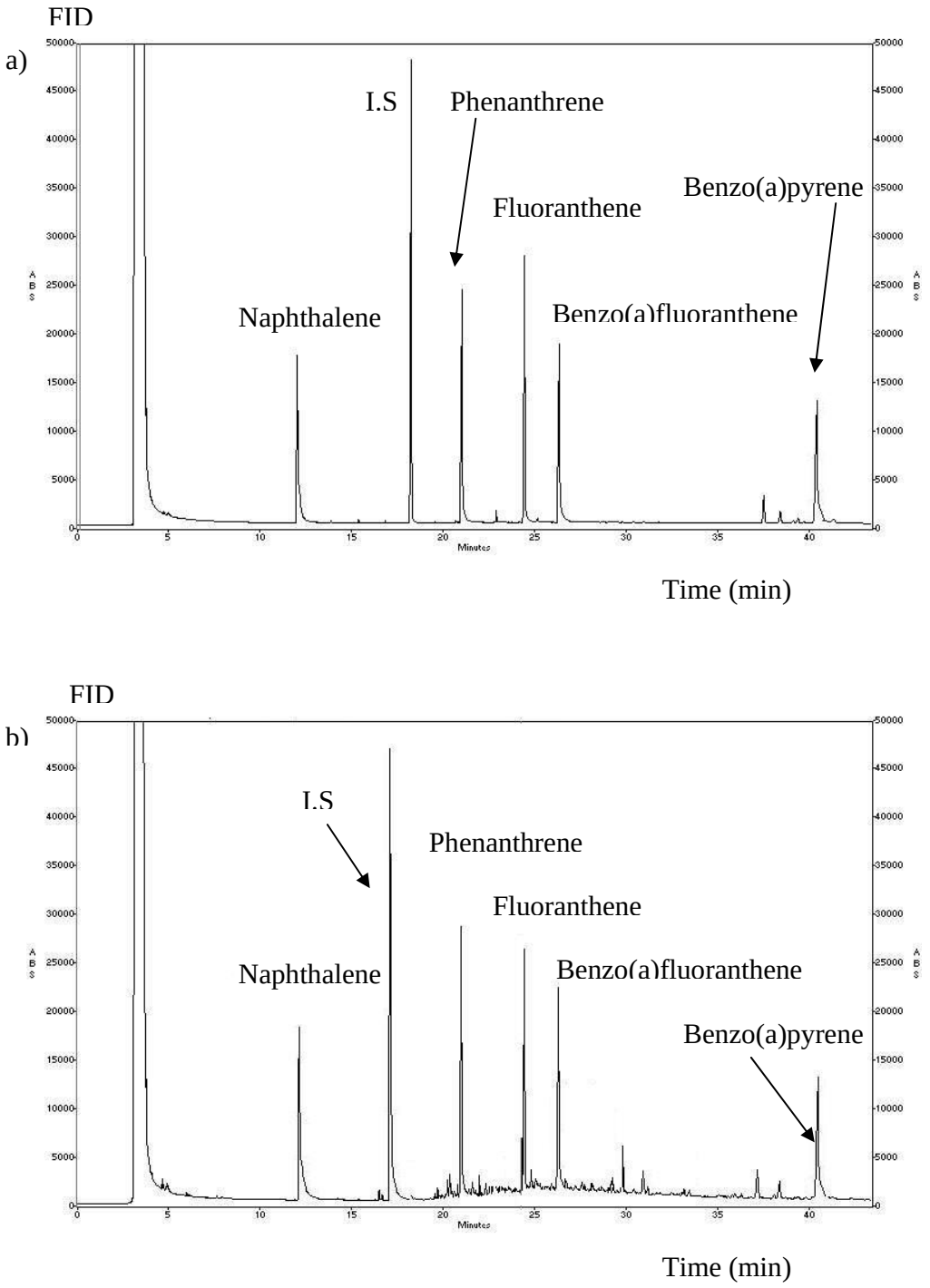


Figure 6.4 GC spectra for PAH extract clean-up using (a) Silica column and (b) SPE silica cartridge.

6.5 Identification of PAHs

After the extraction method and extract clean-up of PAH from the sludge sample was developed, the determination of PAH in real samples was carried out. Sewage sludge samples have a variety of organic pollutants, hence peak confirmations of PAH in real samples were performed using retention time, co-injection technique, Kovats retention index and GC-MS techniques.

6.5.1 Identification of PAH Based On Retention Time

Qualitative information for PAH can be obtained using retention time analyte. Using the same capillary column and temperature programming for PAH separation, the analyte in sample can be identified based on its retention time. In this study, retention time for PAH analyte in sludge sample have slight similar retention time with the standard PAH. Table 6.4 showed the retention time for five PAH standards and PAH analyte in the real samples. Whereas, Figure 6.5 until Figure 6.7 showed the GC profile for sludge sample from different location.

Table 6.4 Identification of PAH based on retention times.

Analyte	Retention time (minutes)			
	Standard PAH	LK	SA	KT
Naphthalene	12.058	12.017	12.070	12.042
Phenanthrene	21.075	TD	TD	21.063
Fluoranthene	24.517	TD	TD	TD
Benzo(a)Fluoranthene	26.400	TD	TD	TD
Benzo(a) pyrene	40.508	TD	TD	TD
I.S	18.308	18.277	18.326	18.286

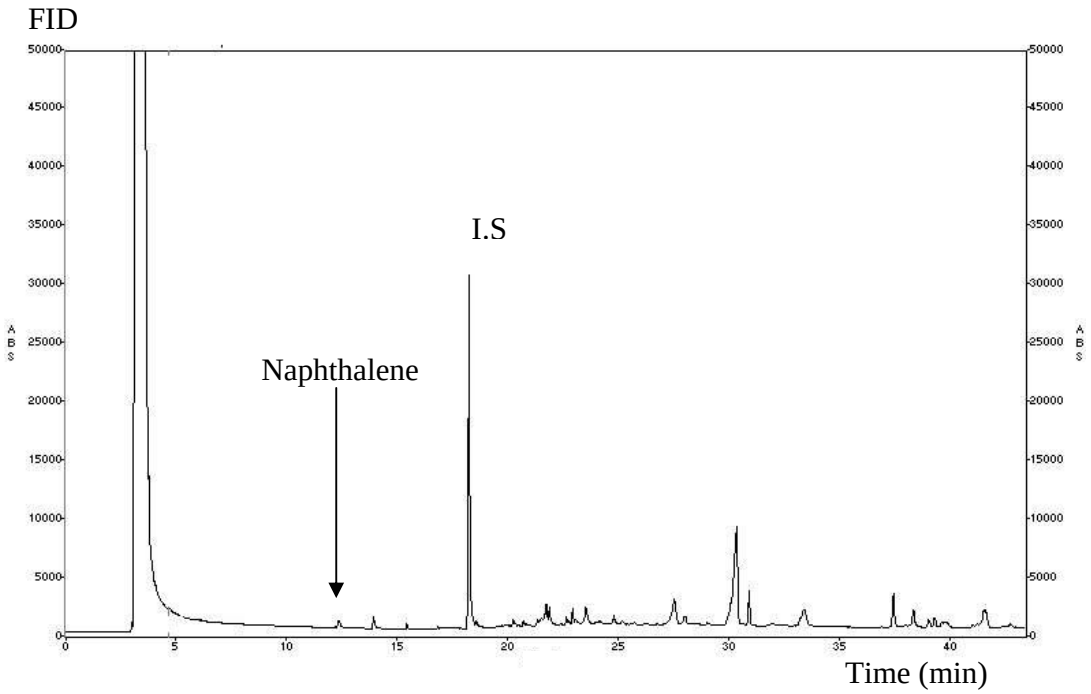


Figure 6.5 GC chromatogram for LK sample.

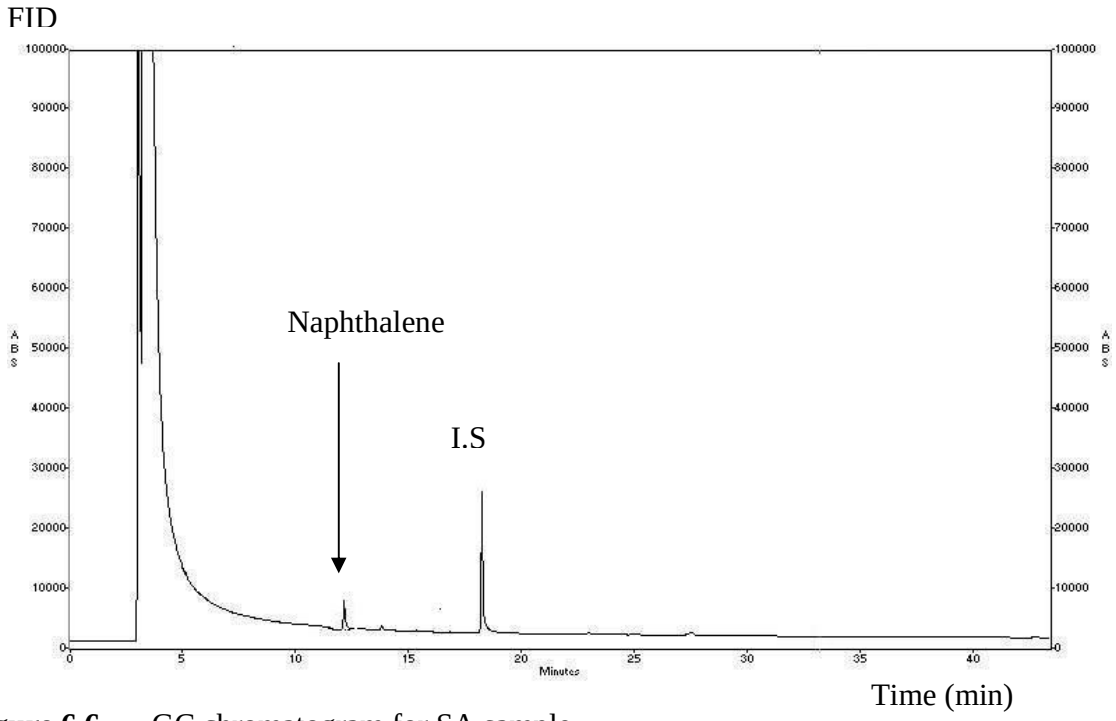


Figure 6.6 GC chromatogram for SA sample.

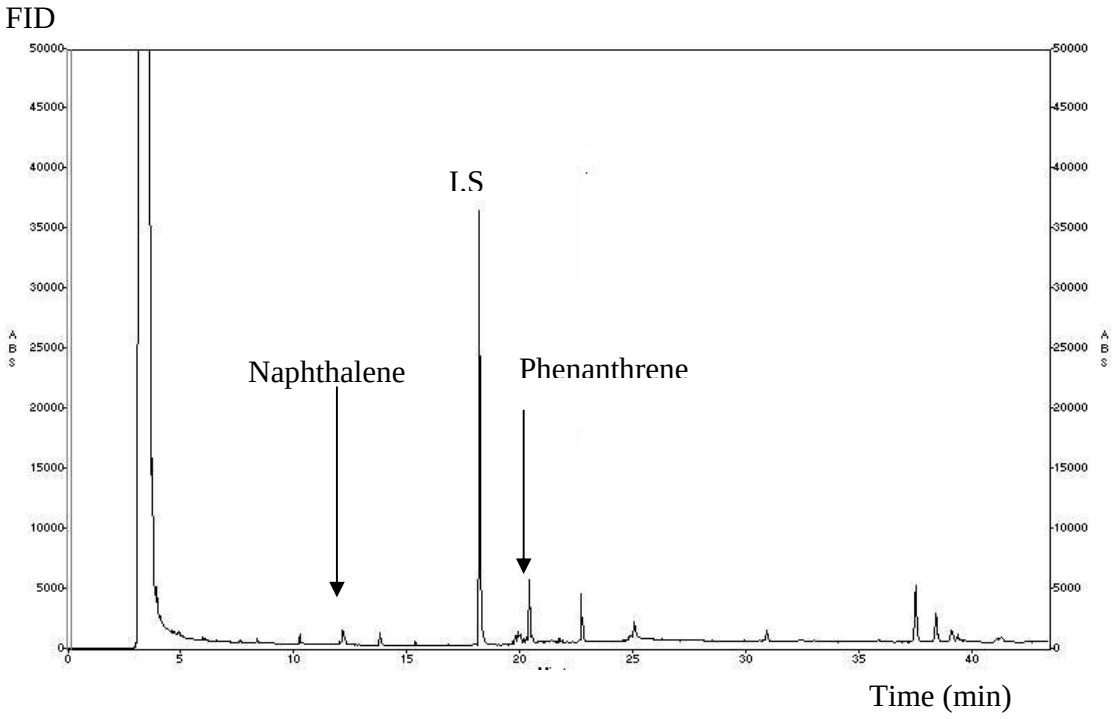


Figure 6.7 GC chromatogram for KT sample.

From the three GC profiles above, it can be shown that silica column was more efficient for extract sample clean-up due to less interference peak in the GC chromatogram. Beside that, the GC profiles above also showed that the LK and KT samples may contained other organic pollutants compared to the SA sample. Therefore confirmatory measure was carried out using co-injection and GC-MS techniques.

6.5.2 Identification using Co-injection

Co-injection is one of the techniques often used for qualitative analysis in GC. Co-injection is a technique in which a small volume of the standard sample is injected together with the sample extracts. The peak area of the suspected peak at the same

retention time will tend to increase. In this study, co-injection technique was used to confirm the existence of PAH in the sludge sample. Comparison using retention time of PCBs is not accurate because there might be other contaminants in the sample which happened to have the same retention time as the PAH standards under study.

Standard naphthalene solution was added to the sample extract LK and SA whereas the standard solution of naphthalene and phenanthrene was added to KT sample. From the chromatograms of co-injection, the concentration of these PAH were totally increased, the peak area for these PAH compounds were significantly increased compared to the chromatogram prior to co-injection (Appendix F).

6.5.3 Identification using Retention Kovats Index

Kovats index (I) is retention index based on the homolog series *n*-alkane. Each of the *n*-alkane is given a value index 100z, where z is the carbon number. Kovats Retention index for each compound can be obtained from the graph based on the t_R value for these compound. For the determination of PAH in sludge samples, PAH peaks were confirmed by comparison with the Kovats retention index standard. Graph of retention time versus carbon number was plotted for *n*-alkanes $C_{10}H_{22}$, $C_{12}H_{26}$, $C_{14}H_{30}$, $C_{16}H_{34}$, $C_{18}H_{36}$, $C_{20}H_{42}$, $C_{22}H_{46}$, $C_{24}H_{50}$, $C_{26}H_{54}$, $C_{28}H_{58}$ and $C_{30}H_{62}$ (Figure 6.8). This graph was plotted based on the *n*-alkane mixture using the same temperature programming for GC.

Linear equation was obtained from Figure 6.8;

$$Y = 0.0269x - 13.102$$

$$\text{Hence, } x = \frac{y + 13.102}{0.0269} \qquad (6.2)$$

$$R^2 = 0.9869$$

With, y is retention time sample, x is Kovats retention index and R^2 is correlation coefficient

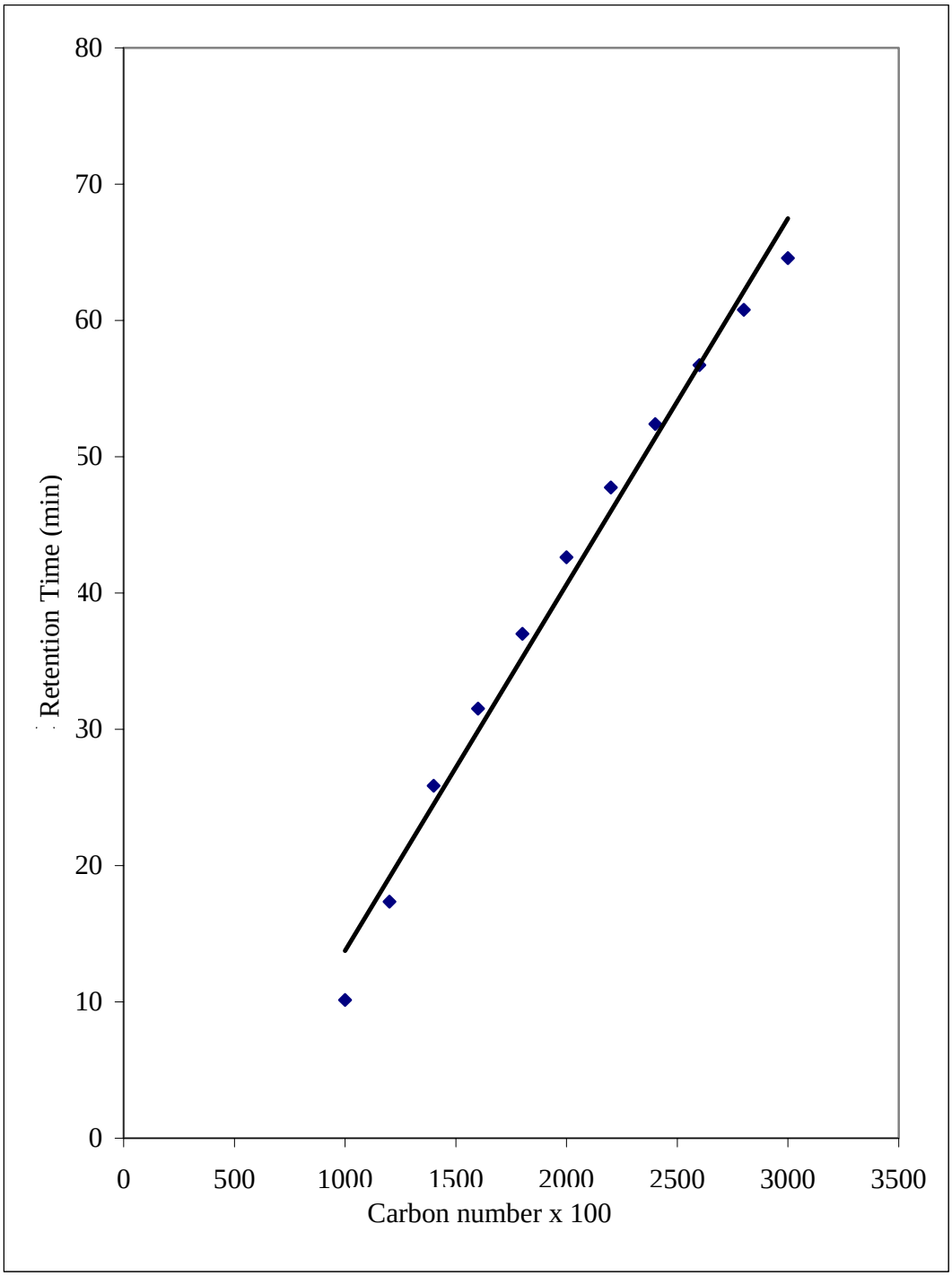


Figure 6.8 Graph of retention time versus carbon number x 100 for standard hydrocarbon mixture.

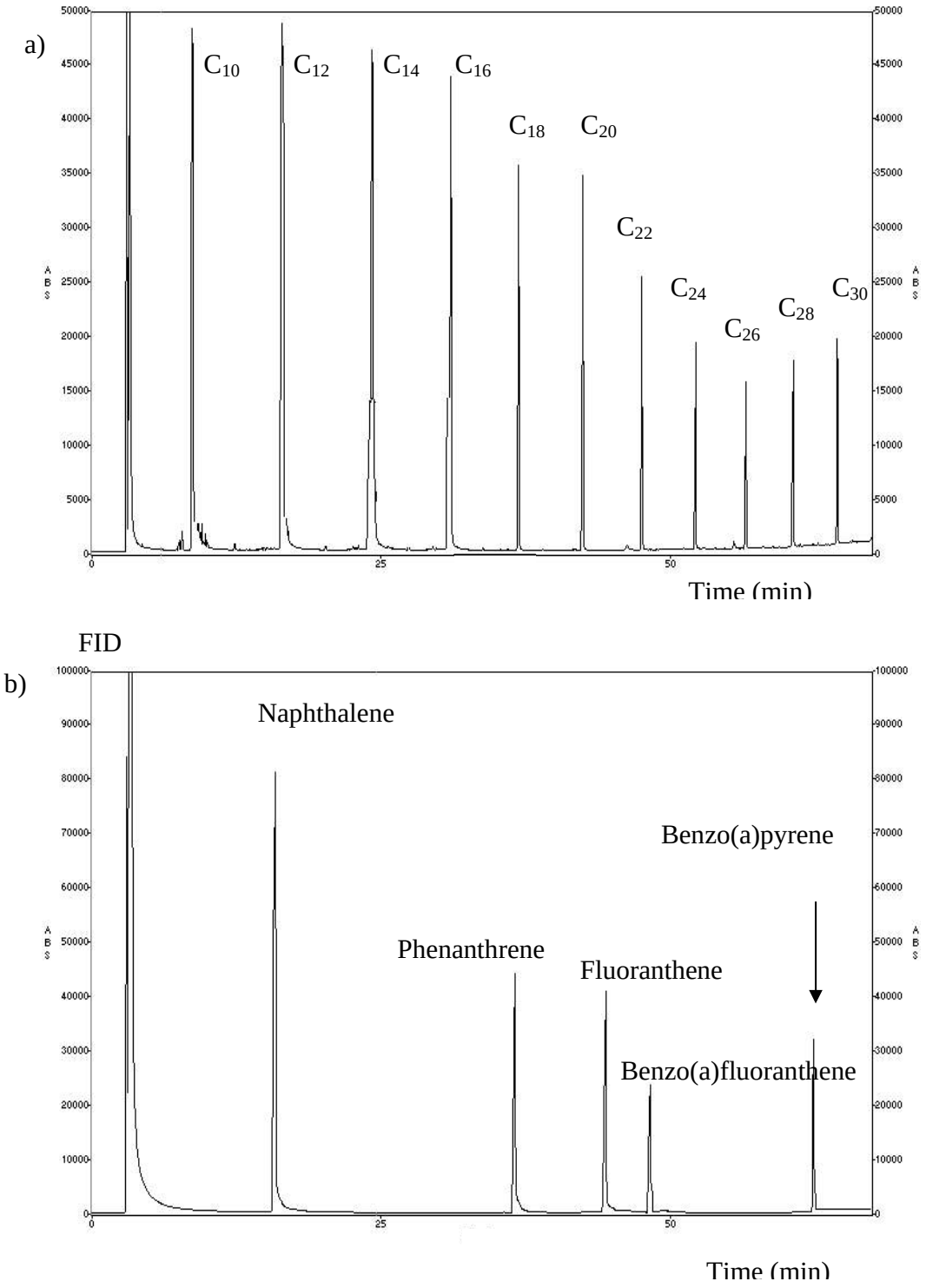


Figure 6.9 GC chromatograms for (a) *n*-alkane mixture C_{10} – C_{30} and (b) Five PAH standard mixture.

In order to obtain the Kovats retention index of naphthalene and phenanthrene for LK, SA and KT samples, calculation was carried out using the Equation 6.2.

Table 6.5 Comparison of Kovats retention index between standard PAH, PAH from real sample and previous study.

	Retention Kovats index				
PAH	Standard PAH	LK	SA	KT	Previous study *
Naphthalene	1080	1085	1086	1081	1172
Phenanthrene	1849	-	-	1851	1742
Fluoranthene	2142	-	-	-	2011
Benzo(a)fluoranthene	2284	-	-	-	-
Benzo(a)pyrene	2809	-	-	-	2778

* Ernaert (1979) – Glass column SE 52 (2.5 %) (16.6 m x 0.25 mm ID) Flow rate gas helium 6.0 mL/min. GC Condition: Injection port temperature at 300 ^{o}C and detector temperature at 300 ^{o}C. Temperature programming from 70 ^{o}C (held for 5 minutes) then ramped to 320 ^{o}C at a rate of 4 ^{o}C/min.

In this study, the Kovats retention index was slightly different with previous study due to a few different parameters such as type of stationary phase, length of capillary and carrier gas flow rate. However, the Kovats retention index in this study is applicable for standard reference for future study using the same GC parameter. The Kovats retention index between standard PAH and PAH from the three locations were slightly similar. These confirmed that, the analyte of naphthalene and phenanthrene was present in the sludge samples analysed.

6.5.4 Identification of PAH using GC-MS

GC-MS analysis was carried out for identification of PAH residue in sludge sample. Figure 6.10, Figure 6.11 and Figure 6.12 showed the total ion chromatogram for LK, SA and KT samples respectively. Figure 6.13 and Figure 6.14 showed the comparison between mass spectrum of naphthalene and phenanthrene from Wiley data base with mass spectrum from the sample respectively. Mass spectrum for naphthalene and phenanthrene showed that the corresponding m/z peaks of 128 and 178 for molecular mass. Both of Figures when compared with the mass spectrum of naphthalene and phenanthrene from the Wiley data base gave probability greater than 90 %. This result confirmed the presence of naphthalene and phenanthrene in the Soxhlet extract. Besides that, this research also indicated the successful clean-up of the sample extract.

However, the chromatogram also showed another peak, which may be due to the presence of other organic contaminants in sludge. The identification of the unknown peak was also carried out. However, it's mass spectrum gave lower probability matching when compared with the Wiley data base.

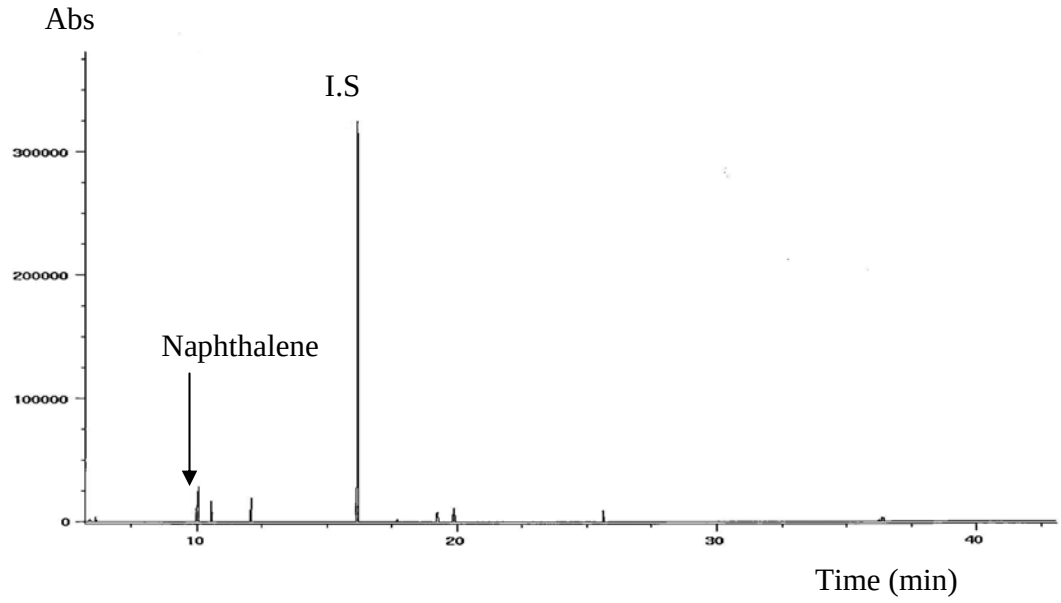


Figure 6.10 Total ion chromatogram for LK sample.

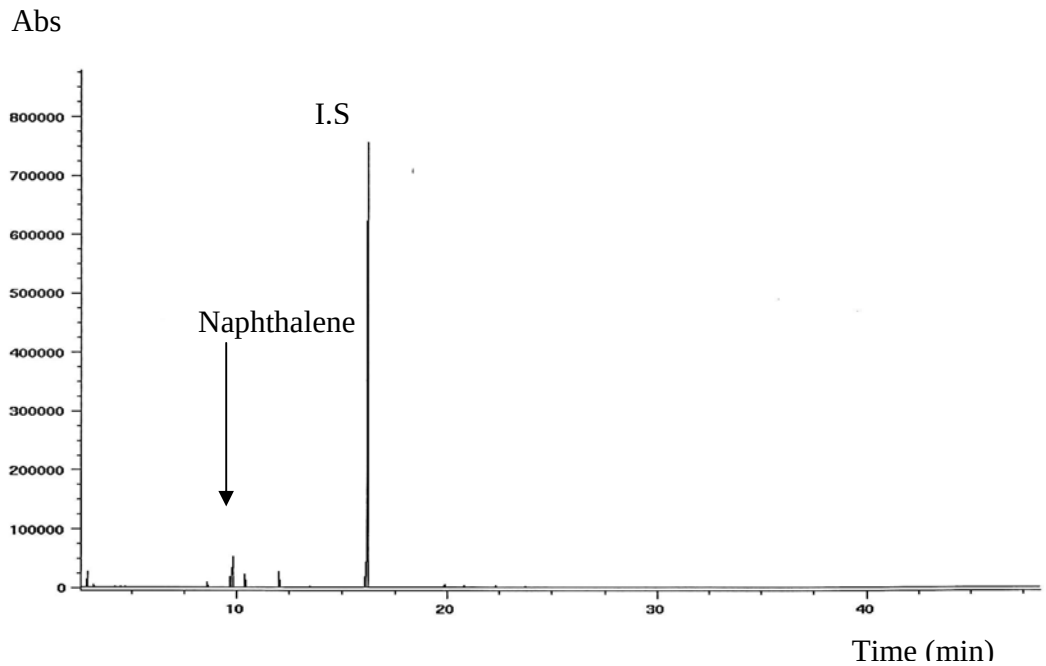


Figure 6.11 Total ion chromatogram for SA sample

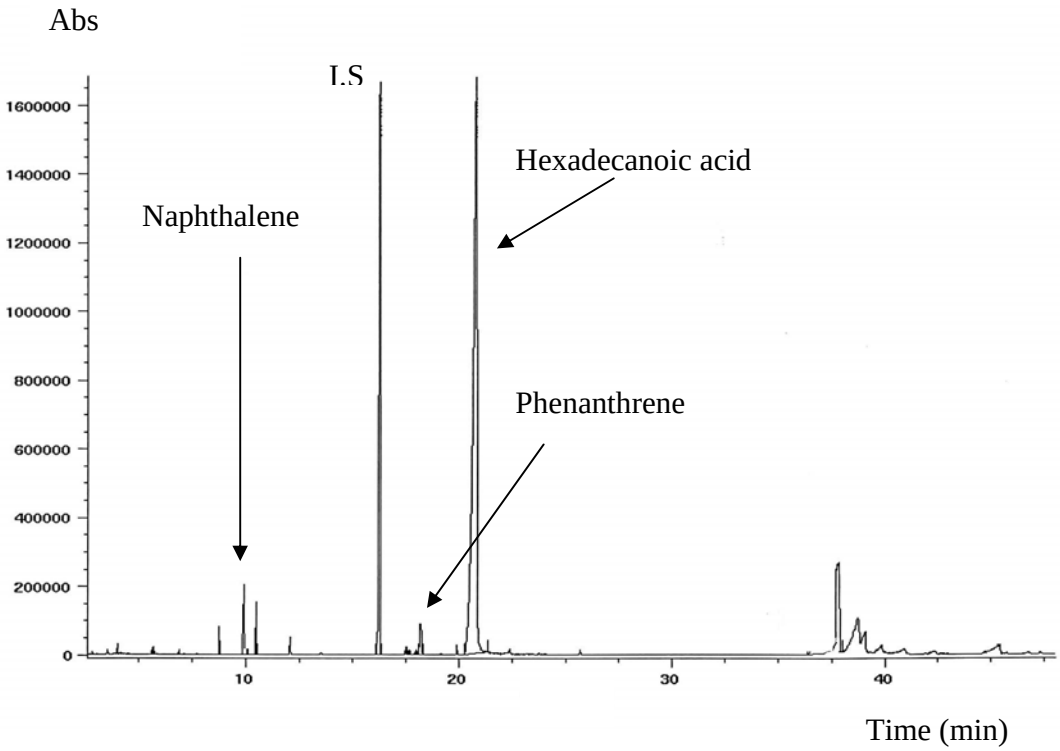


Figure 6.12 Total ion chromatogram for KT sample

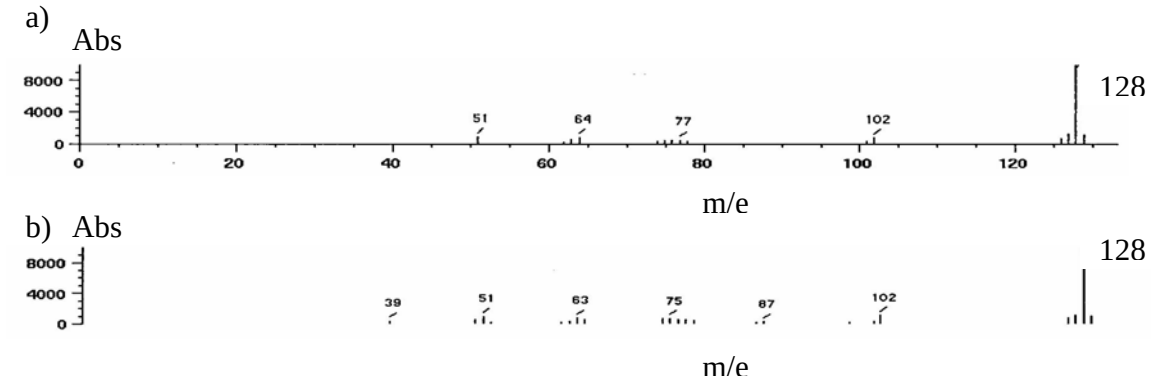


Figure 6.13 Comparison of mass spectrum of naphthalene (a) from Wiley data base and (b) real sludge sample.

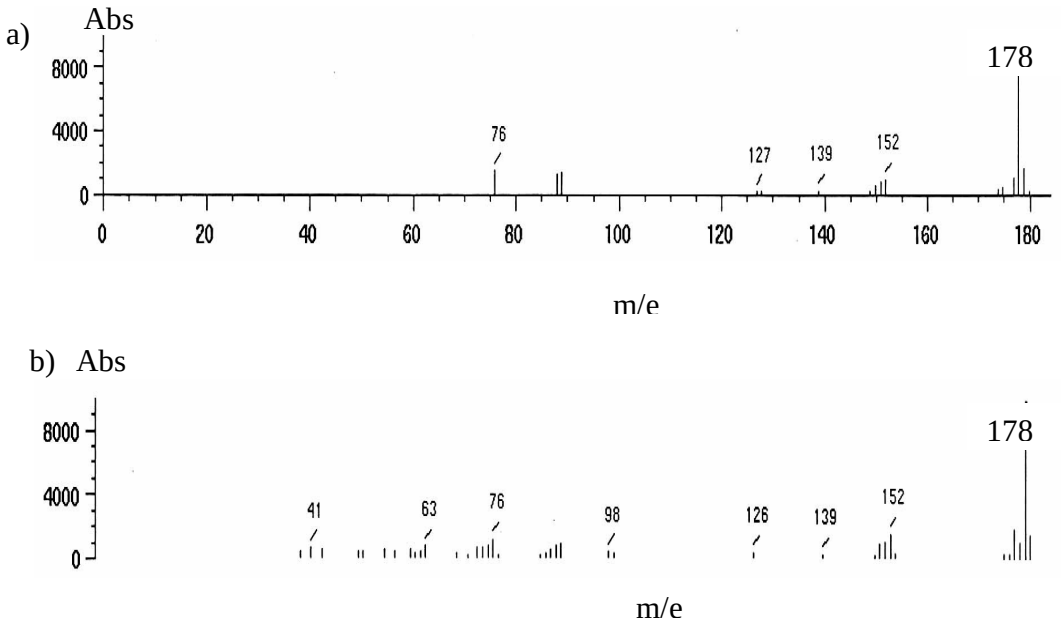


Figure 6.14 Comparison of mass spectrum of phenanthrene (a) From Wiley data base and (b) Real sludge sample.

6.6 Analysis of PAH from Three Location

The sampling area in this study was divided into two categories; they are light industrial area and a combination of light industry with domestic area. These areas were chosen in order to carry out a comparison study between sludge from industry and domestic areas. In this study, sludge sample from three different locations were sampled and taken back to the lab for analysis. After pretreatment of the sludge, these sludges were extracted using Soxhlet extraction and extract clean-up with silica column was carrier out. The PAH residues were analysed using GC-FID. Table 6.6 showed the concentration values of PAH residue found in the sludge samples analysed.

Table 6.6 Concentration of PAH residues in three different locations.

PAH	Concentration (mg/kg)				
	LK	SA	KT	Previous study*	Maximum limit (EU)#
Naphthalene	5.38 ± 0.13	5.46 ± 0.20	4.55 ± 0.17	1.12	6
Phenanthrene	ND	ND	4.07 ± 0.08	1.82	
Fluoranthene	ND	ND	ND	0.42	
Benzo(a) fluoranthene	ND	ND	ND	NR	
Benzo(a)pyrene	ND	ND	ND	0.21	

*Rechcigl, (1995)
ND = Not detected
NR = Not Reported
# Total of Naphthalene, Phenanthrene, Fluoranthene, pyrene, benzo(b+j+k) Fluoranthene, benzo(a) pyrene, dan indeno(1,2,3-cd)pyrene.

Based on the above Table, LK and SA samples showed the presence of naphthalene with concentration 5.38 mg/kg and 5.46 mg/kg respectively. Other PAHs were not detected in this study. For KT sample, naphthalene and phenanthrene were

detected with concentration of 4.55 mg/kg and 4.07 mg/kg respectively. Other PAHs not found in this study could be due to the lower concentration and/or out of the range of detection for the GC instrument.

Sources of the PAH in sludge sample could come from industrial and domestic activities. Industrial activities that produced PAH may come from gas factory, aluminum and metal industry, oil and plastic industry. Sources from domestic may be derived from cooking oil frequently used in the kitchen. Sources of naphthalene for LK and SA samples could be originated from industries such as metal factory and mechanical industries. Whereas, metal production factory, concrete making industry, and plastic factory and forge industry are primary sources of PAH in the SA sample. KT sample was slightly different due to the presence of naphthalene and phenanthrene detected in the samples. Both naphthalene and phenanthrene may be derived from industry and domestic activity such as metal production industry, plastic factory and forge industry, whereas sources of PAH from domestic could have come from cooking oil. KT sample showed the higher concentration with 8.63 mg/kg followed by SA and LK samples.

Sewage sludge in this study found to have PAH due to during wastewater treatment, more of the PAH have characteristic of no charges (neutral), not dissolved in water and absorbed in solid particle. Percentage of solid (65 % to 85 %) and percent of volatile (16 % to 35 %) was high in this study, indicatating that these sludge samples have more total solid particle to absorb PAH compounds. Previous study showed that when PAH is absorbed in solid particle, interaction between PAH molecule with solid particles are strong.

Concentration of naphthalene and phenanthrene in three locations were 4 times higher than previous study due to this sample being in an area near the industrial activity and combustion activity. Combustion activity is one of major sources produced of PAH. In this study, the concentrations of PAHs were below the standard limit of PAH in sludge (EU).

6.7 Summary

This study was successful in developing suitable method for analysis of PAH in sewage sludge. Based on the GC-FID parameter, the separation of PAH mixture was satisfactory. Recovery studies showed that both procedures gave quantitative recoveries of PAH, however silica gel column was evidently more efficient than the SPE silica cartridge.

Several techniques for PAH analyte identification were carried out such as comparison of retention time, co-injection technique, comparison with Kovats retention index and using GC-MS. Comparison of concentration of PAHs in three different location was carried out. Higher concentration of PAH was obtained from KT sample compared to the other samples due to the KT sample being near the industrial and domestic activity area.

CHAPTER 7

ANALYSIS OF ORGANOCHLORINE PESTICIDES

7.1 Chromatographic Separation of OCP mixture

The standard OCP mixture solution consisting of five compounds, namely lindane, dieldrin, TDE, pp-DDT and heptachlor was separated using GC-ECD on a BPX 5 capillary column. Based on Figure 7.1, all the OCP analytes were successfully separated, with a total time of less than 11 minutes. Chloropyfos was used as internal standard and eluted between heptachlor and dieldrin.

7.2 Limit of Detection for OCP

In order to determine the sensitivity of the technique, the limit of detection (LOD) of each OCP was determined by the analyte signal being three times the background signal. Detection limits for the OCPs are as shown in Table 7.1.

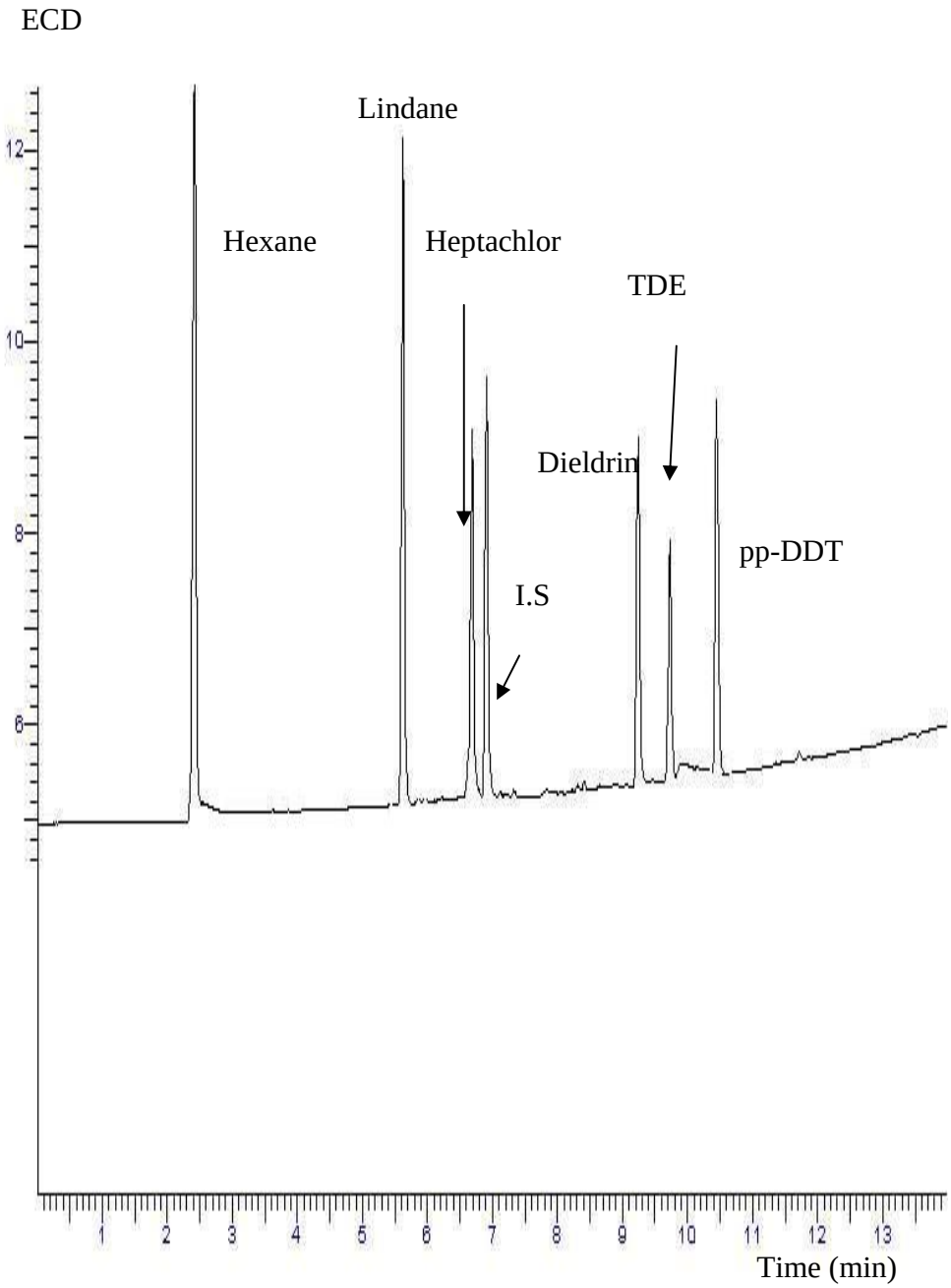


Figure 7.1 GC-ECD separations of five OCPs using a BPX 5 capillary column. GC Conditions: Injection port temperature at 250 ^{o}C and detector temperature at 340 ^{o}C. Temperature programming from 200 ^{o}C (held for 3 minutes) then ramped to 340 ^{o}C (held for 3 minutes) at a rate of 10 ^{o}C/min.

Table 7.1 Comparison of limit of detection for OCPs

	Limit of detection (μg/kg)	
OCP	In this study	Previous study *
Lindane	39.0	0.05
Heptachlor	7.8	0.10
Dieldrin	39.0	0.06
TDE	7.8	0.10
pp-DDT	7.8	0.05

*Chee *et al.*, (1996)
Previous study utilized a GC-SIM (*selective ion monitoring*)-MS with HP-5MS capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness) GC Conditions: Temperature programming from 100 ^{o}C (held for 1 minutes) then ramped to 170 ^{o}C at a rate of 25 ^{o}C/min and 270 ^{o}C at a rate of 2 ^{o}C/min increased to 290 ^{o}C at a rate 25 ^{o}C/min (held for 3 minutes) Temperature injection, interface and sources of ion at 270 ^{o}C, 280 ^{o}C and 200 ^{o}C respectively. Helium gas at 1.29 mL/min.

The LOD for the seven OCPs shown were generally higher compared to other study (Chee *et al.,* 1996). The value of LOD in this study is applicable for analyses of real samples because the OCP residue level in sewage sludge are generally in the range between 0.72 to 209.09 mg/L (Rechcigl, 1995).

7.3 Calibration Graph of Standard OCP

In order to obtain the concentration of OCP in real samples, the soxhlet extract (50 μL) was added with 50 μL chloropyfos (I.S.) prior inject to GC-ECD. Figure 7.2 shows the calibration graph for each OCP in this study whereas the linear calibration graphs equation and correlation coefficients for five OCP are shown in Table 7.2.

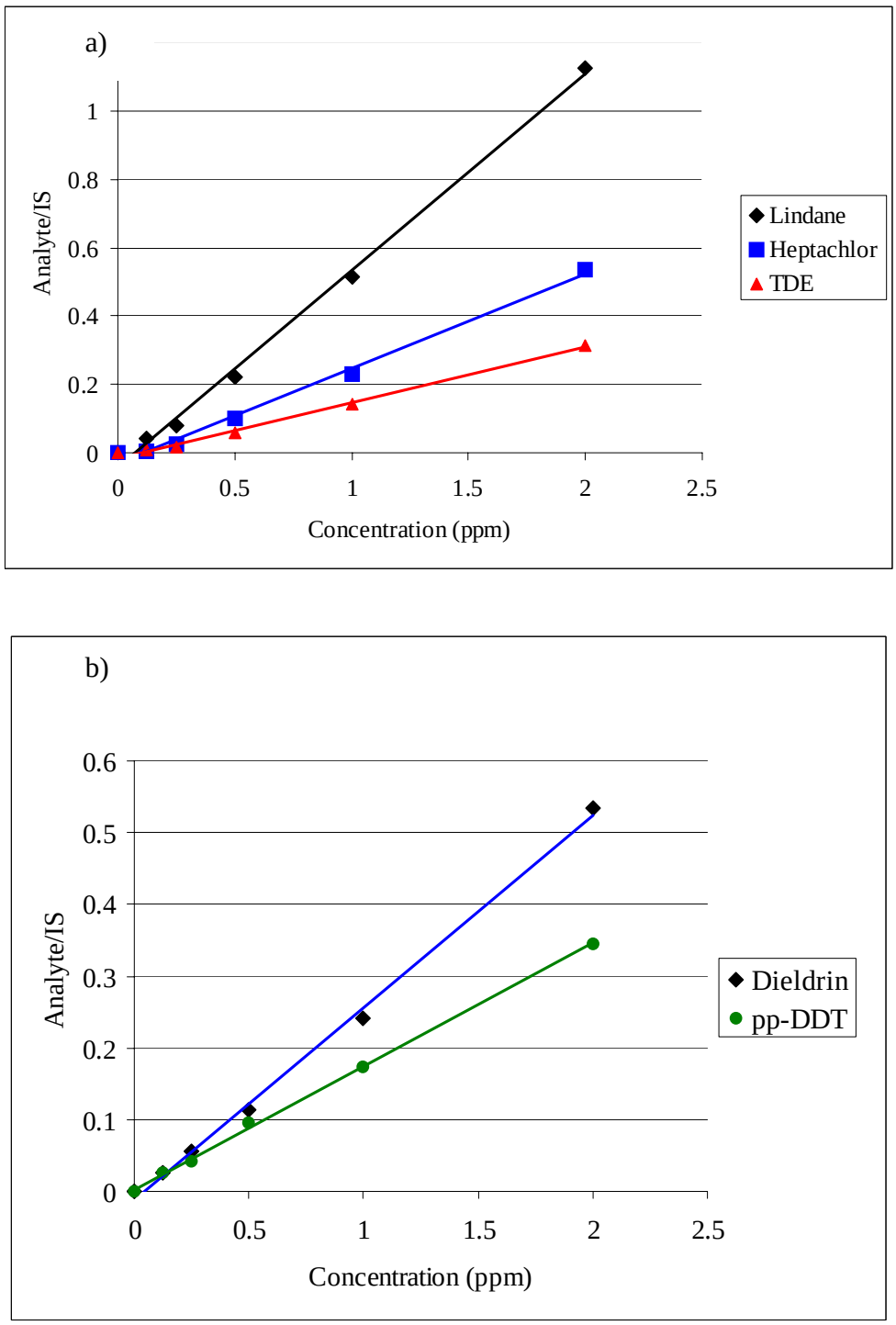


Figure 7.2 Calibration graphs for (a) Lindane, heptachlor and TDE, and (b) Dieldrin and pp-DDT.

Table 7.2 Linear equation and correlation coefficients for each OCP.

OCP	Equation	Correlation coefficients
Lindane	y = 0.5639x - 0.0167	0.9984
Heptachlor	y = 0.2679x - 0.0085	0.9984
Dieldrin	y = 0.2708x - 0.0031	0.9988
TDE	y = 0.1543x - 0.0011	0.9979
pp-DDT	y = 0.0321x - 0.0092	0.9977

Concentration OCP (µg/g) = value OCP from linear equation (µg/mL) x 2 (7.1)

$$\text{x 1 mL x } \frac{1}{10g}$$

7.4 Recovery Study of OCP

The recovery study of OCP was carried out in order to evaluate the efficiency of extraction method of OCP in sewage sludge using GC-ECD. Few reports have appeared in previous years regarding the recovery study of OCP in solid sample (Tan and Vijayaletchumy, 1994; Snyder *et al.*, 1992). In this study, recovery studies of OCP were conducted using spiked blank sludge and were extracted in triplicates using Soxhlet extraction. The soxhlet extract greatly required an extract clean-up prior to analysis using GC-ECD. In this study, focused was on the extract clean-up using silica column technique. Table 7.3 shows the comparison between percentage recovery of OCP in this study and previous study using Soxhlet extraction method.

Table 7.3 Comparison of percentage recovery of OCP in this study with previous study (Tan and Vijayaletchumy, 1994)

	Recovery (%)	
OCP	In this study	*Previous study
Lindane	87.96 ± 4.74	97.0 ± 6.6
Heptachlor	87.06 ± 7.55	95.0 ± 7.5
Dieldrin	85.55 ± 3.85	94.0 ± 9.6
TDE	89.54 ± 5.62	Not reported
pp-DDT	82.09 ± 5.34	96.0 ± 8.6

* Soxhlet extraction using mixture of 250 mL hexane and acetone (1:1) for 10 hour. Water river sediment was used.

Based on the above table, the results showed that the percentage recovery for all five OCPs were superior with more than 80 %. TDE showed the highest percentage recovery followed by lindane, heptachlor, dieldrin and pp-DDT whereas the standard deviations for all five OCPs were in the range of 3 to 8 %. However, the comparison with previous study showed that the soxhlet extraction method using silica column clean-up were suitable for determination of OCP in sewage sludge. The effectiveness and efficiency of silica column clean-up can be seen from the GC-ECD chromatogram that showed less interference peaks (Figure 7.3).

7.5 Identification of OCP

There are several methods that have been used for identification of OCPs in real sewage sludge sample. The common methods used for identification of analytes are comparison with retention time, co-injection technique and GC-MS.

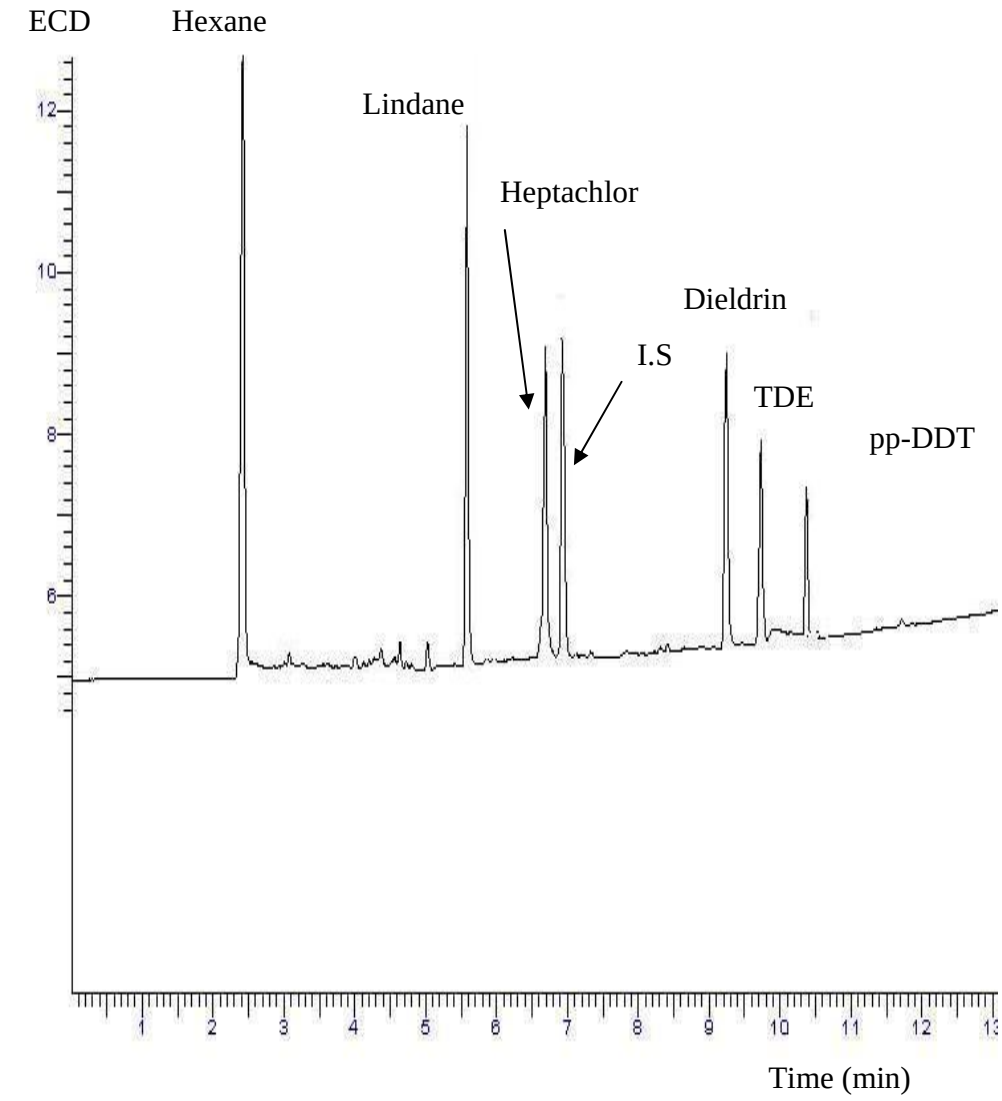


Figure 7.3 Chromatogram of Soxhlet extract for spike sample.

7.5.1 Identification of OCP Based on Retention Time

One of the methods used for confirmation of OCP in sewage sludge was the comparison retention time of OCP with retention time of the standard. Table 7.4 shows

the retention time of OCP with retention time of standard, whereas Figure 7.4, 7.5 and 7.6 show the GC spectra for three different locations.

Table 7.4 Comparison of the retention time standard of OCP and OCP in real sample (BPX 5 Capillary column).

	Retention time (min)			
	Standard OCP	LK	SA	KT
Lindane	5.62	ND	ND	ND
Heptachlor	6.69	ND	6.65	6.69
Dieldrin	9.24	9.22	ND	9.24
TDE	9.73	ND	ND	ND
pp-DDT	10.46	ND	ND	10.45
IS	6.91	6.88	6.85	6.90

ND- not detected

Figures 7.4 to 7.6 showed that the silica column was efficient for use as an extract clean-up and less interference peaks in the GC chromatogram was obtained. LK and SA samples were found to contain dieldrin and heptachlor respectively whereas KT sample contained dieldrin, pp-DDT and heptachlor. The confirmation of OCP using other methods was also carried out.

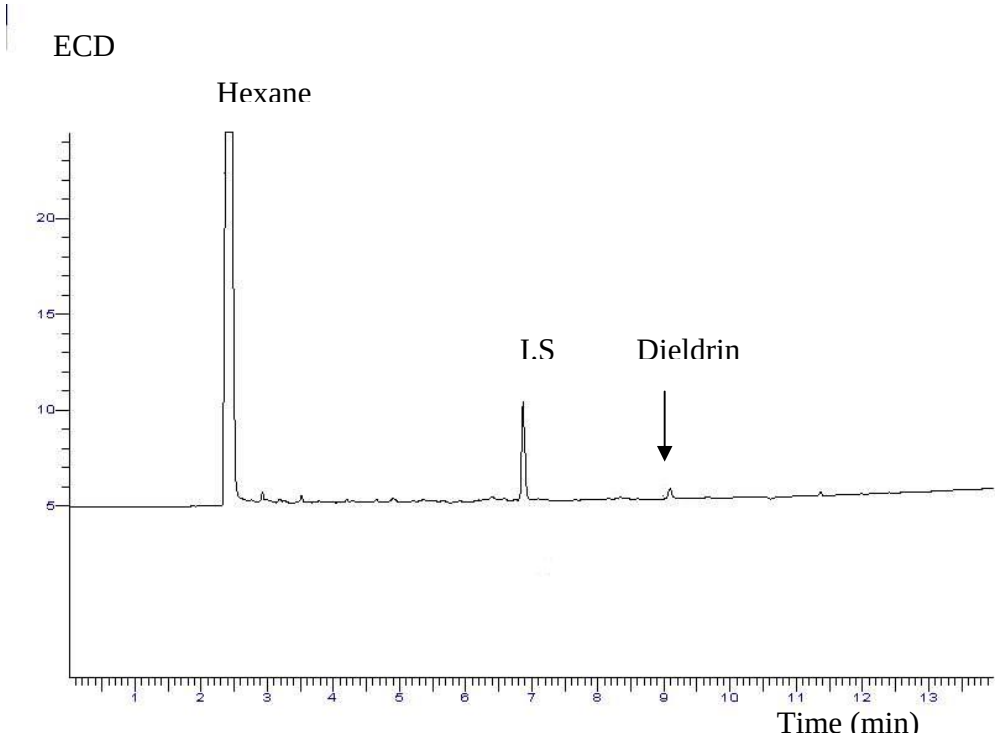


Figure 7.4 GC-ECD chromatogram for OCP in LK sample.

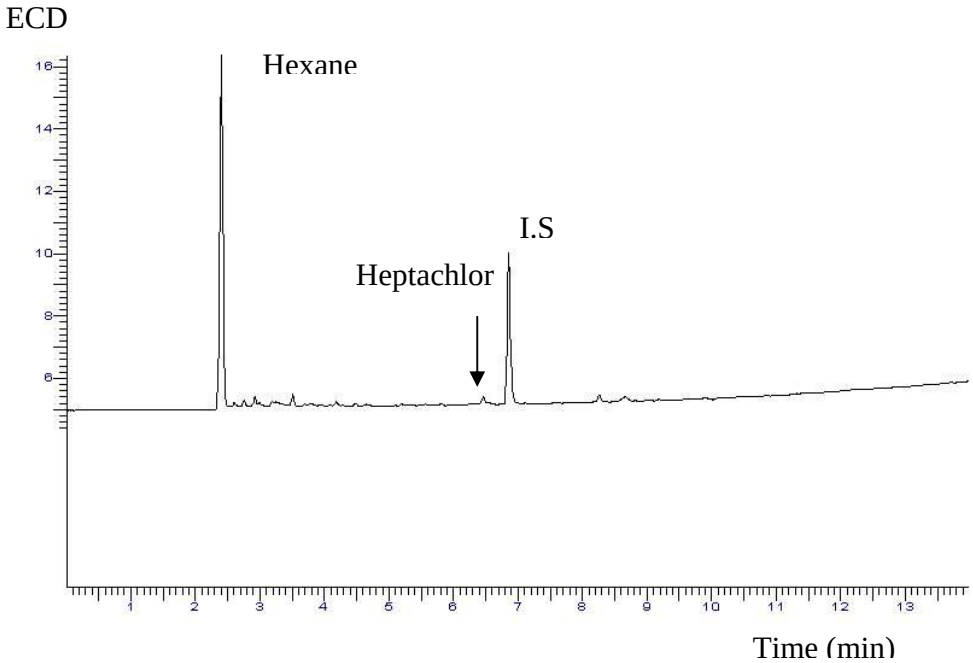


Figure 7.5 GC-ECD chromatogram for OCP in SA sample.

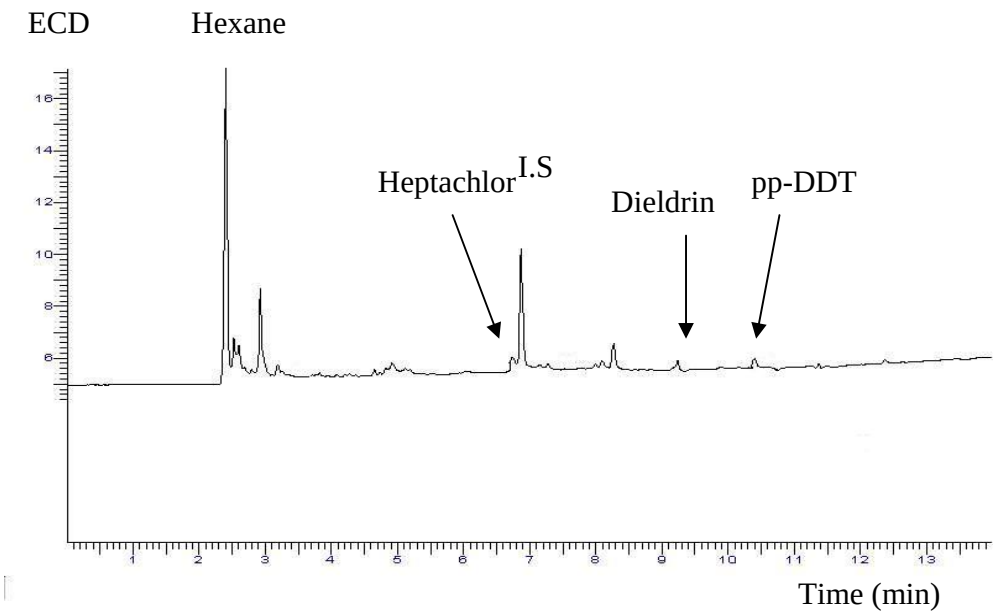


Figure 7.6 GC-ECD chromatogram for OCP in KT sample.

7.5.2 Identification of OCP with Co-Chromatography Technique

The OCP peaks obtained from the GC chromatogram were small. Hence, the further identification was carried out to confirm the OCP residue. Comparison using retention time of OCP is not accurate because there might be other contaminants in the sludge sample. The next confirmatory tests of OCP in sludge sample were via co-injection technique and analysis using different capillary column.

7.5.2.1 Analysis using Different Capillary Column

The identity of OCP was confirmed when analyzed using a different capillary column and with a different temperature programming. In this analysis, HP-5MS

capillary was used and the retention time for each OCP standard and I.S was different with results as shown in Section 6.3.4.1 (Figure 7.7).

For confirmation of the presence OCP in sludge sample, the real sludge sample was analyzed using GC-ECD with HP-5MS capillary and at different temperature program. GC chromatograms for the LK sample (Figure 7.8) and SA (Figure 7.9) were found to contain dieldrin and heptachlor respectively. However, GC chromatogram for KT sample revealed that heptachlor, dieldrin and pp-DDT peaks were detected.

Hence, analysis based on another capillary column (HP-5MS capillary) can be use to confirm OCP analytes in sludge sample. The confirmation of OCP in sludge sample was carried out next using co-injection technique.

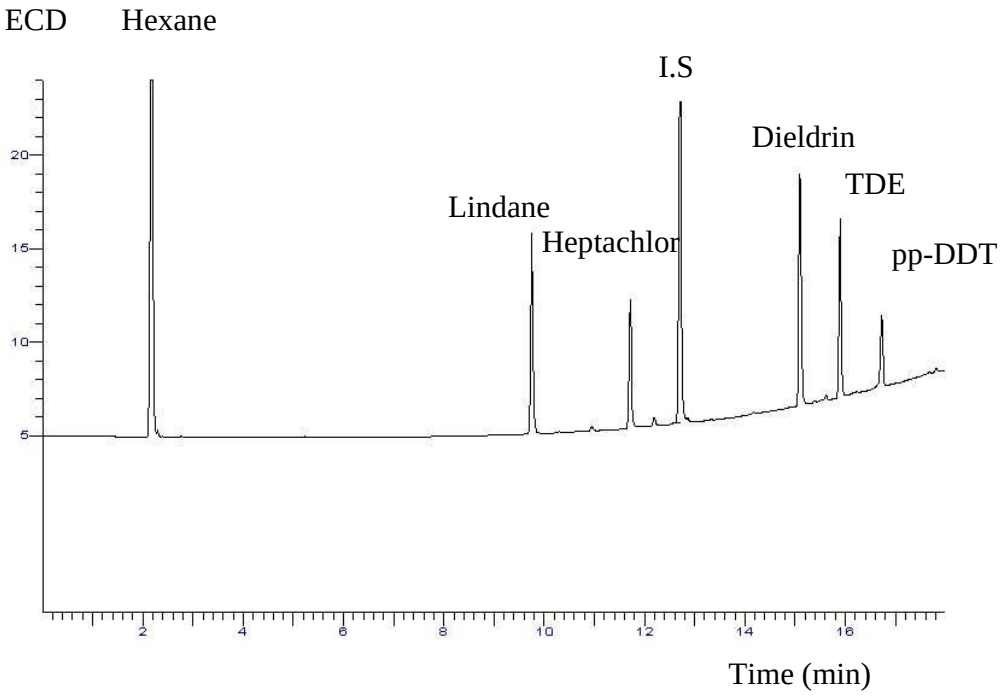


Figure 7.7 Separation of five OCP compounds and I.S using HP-5MS capillary column.

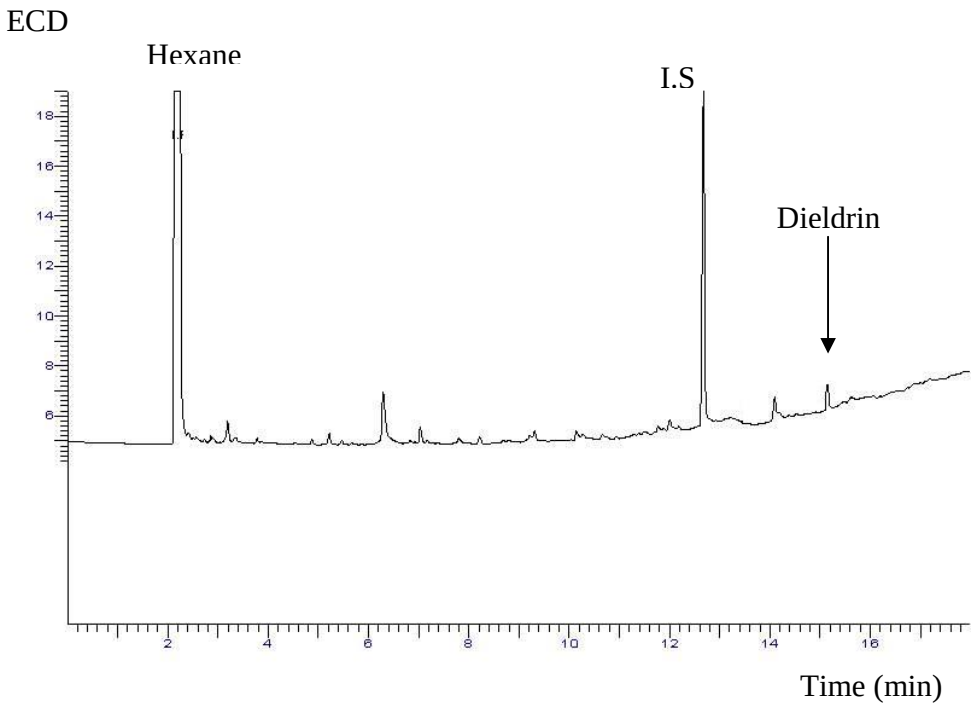


Figure 7.8 GC chromatogram of LK sample using HP-5MS capillary column

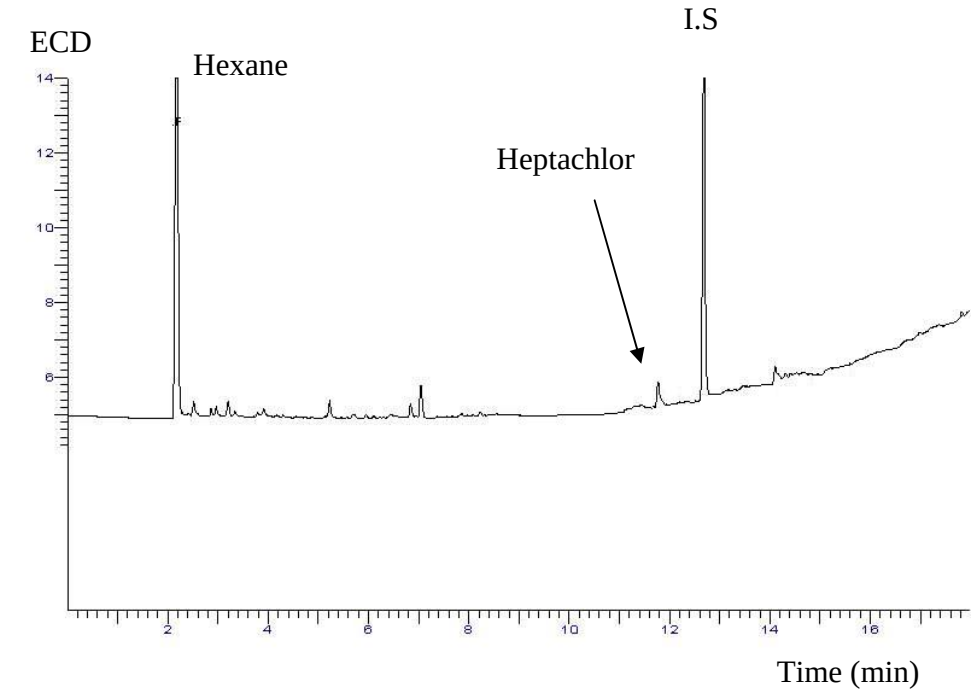


Figure 7.9 GC chromatogram of SA sample using HP-5MS capillary column.

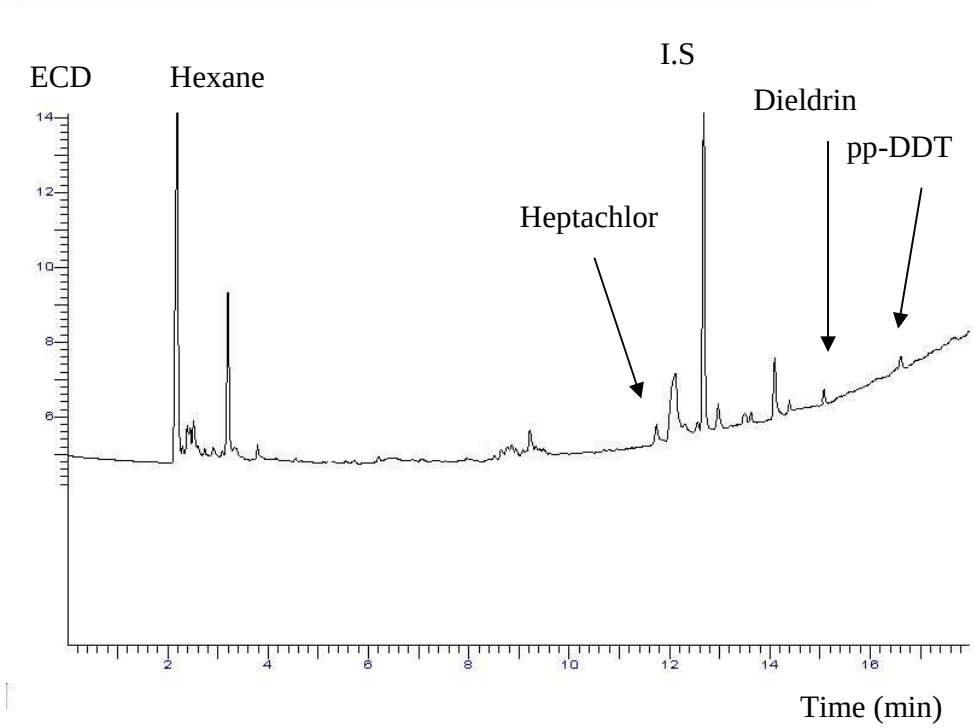


Figure 7.10 GC chromatogram of KT sample using HP-5MS capillary column.

7.5.2.2 Co-injection Technique

For the confirmation of OCP in sewage sludge, three OCP standards (dieldrin, heptachlor and pp-DDT) with concentration of 1 mg/L was spiked into the sample extract prior to GC-ECD analysis. This technique was carried out using the BPX-5 and HP-5MS capillary column. The result showed the GC chromatogram profile for both of dieldrin and heptachlor peaks in the LK and SA samples had their corresponding peak height increased (Appendix G). Whereas the peak for heptachlor, dieldrin and pp-DDT in the GC chromatogram profile for KT sample also showed the increase in peak height. Peak area and height for OCP peaks increased several times compared with the chromatogram before the OCP standard was added.

The results indicated that the peak area and height of OCP peak in both of the capillary columns was increased by several orders of magnitude. Hence, the results confirmed that, the analytes of OCPs were found in this sludge.

7.5.3 Identification of OCP using GC-MS

GC-MS is a powerful instrument for identification of organic molecules in sewage sludge. In this study, GC-MS was used to identify OCPs in sewage sludge due to the powerful technique for confirmation of molecular ion in the sample. Figures 7.11, 7.13 and 7.13 show the total ion chromatogram for LK, SA and KT samples respectively.

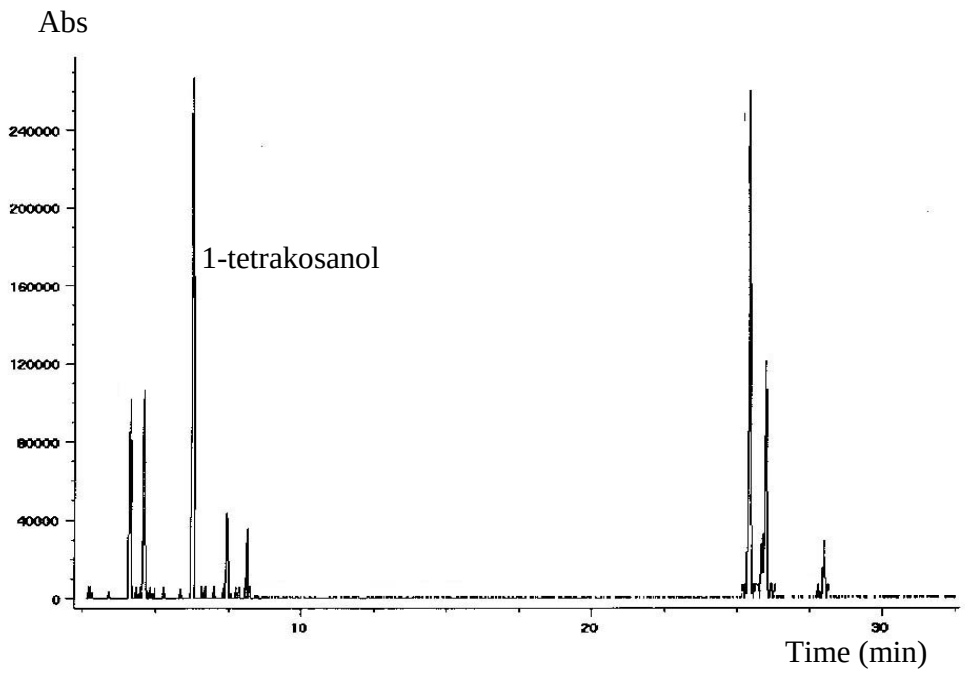


Figure 7.11 Total ion chromatogram of OCP analysis for LK sample.

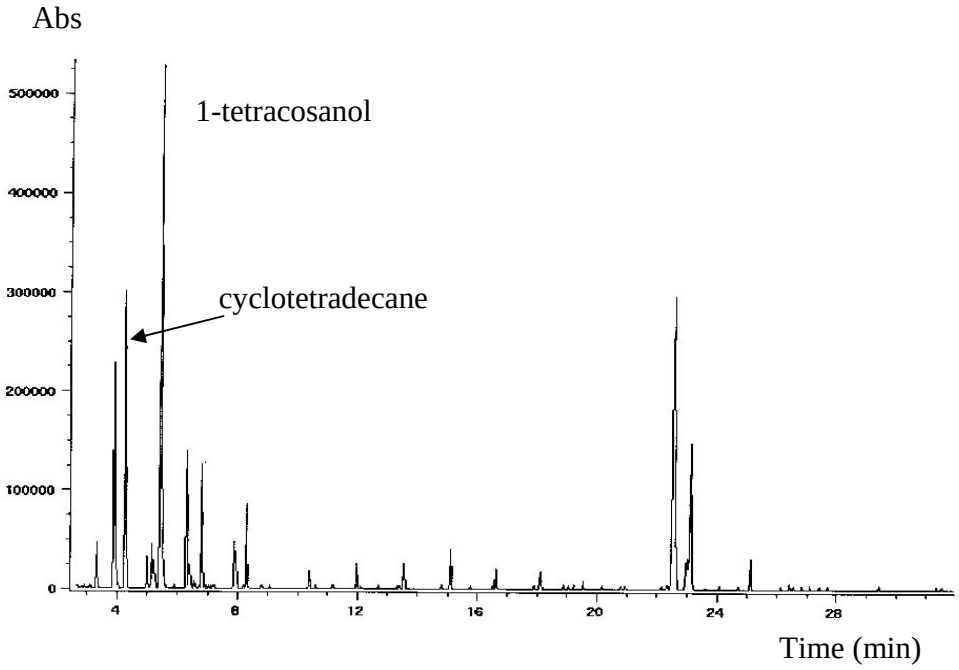


Figure 7.12 Total ion chromatogram of OCP analysis for SA sample.

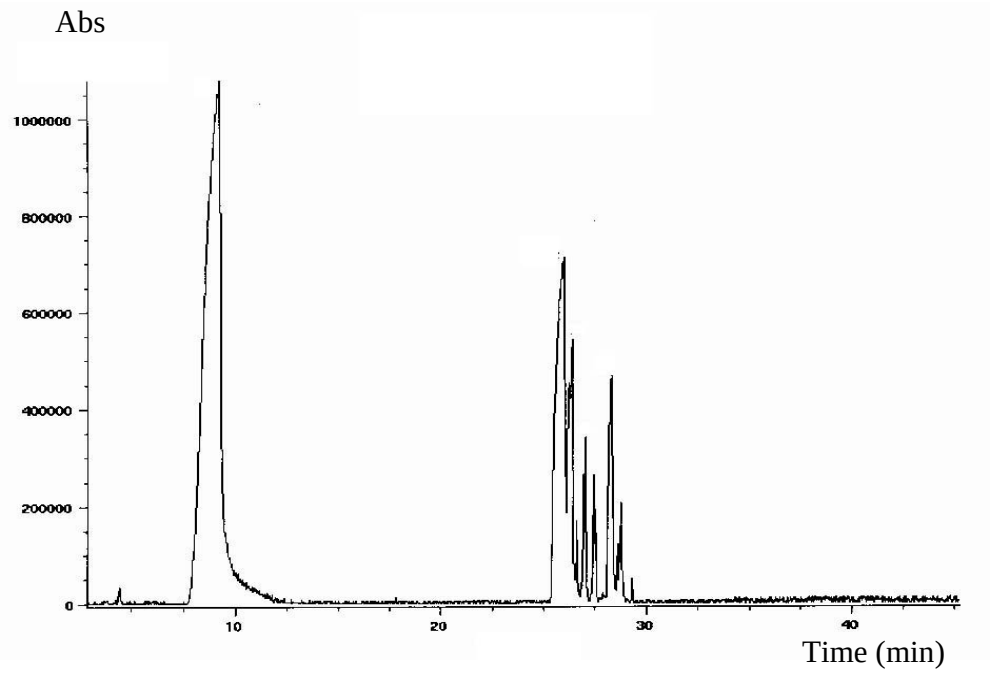


Figure 7.13 Total ion chromatogram of OCP analysis for KT sample.

The total ion chromatogram profile showed that no peak of OCP was obtained. The GC-MS technique could not detect the OCP residue in sewage sludge possibly due to lower concentration of OCP in sludge and also could be due to the concentration OCP being out of the range of the instrument's detection limit. Beside that, the sensitivity of GC-MS in this study was not good compared to that of the GC-ECD. Although the GC-MS instrument could not detect the OCP residue, other organic contaminant were detected showing high probability (>90 %) when compared to the Wiley data base. The GC-MS technique was capable of identifying other organic contaminant in sewage sludge such as 1-tetracosanol and cyclotetradecane.

7.6 Quantitative Analysis of OCP Residue in Sewage Sludge

After the OCPs were successful detected in sewage sludge, quantitative determination of OCP in sewage sludge was carried out. Concentrations of OCPs in the three different locations are shown in Table 7.5.

Table 7.5 Concentration of OCPs in three different sample location.

	Concentration (μg/kg)			
OCP	LK	SA	KT	USEPA Limit
Lindane	ND	ND	ND	209090
Heptachlor	ND	59.48 ± 0.28	61.45 ± 0.47	3180
Dieldrin	51.68 ± 0.26	ND	47.28 ± 0.80	720[a]
TDE	ND	ND	ND	250[b]
pp-DDT	ND	ND	158.97 ± 1.32	250[b]

[a] Total concentration of aldrin and dieldrin
[b] Total concentration of DDT/DDE/DDD
ND = Not detected

Based on Table 7.5, the results showed that the LK and SA samples contained dieldrin and heptachlor residues with concentration of 51.68 µg/kg and 59.48 µg/kg respectively. Three types of OCP were found in KT sample namely heptachlor, dieldrin and pp-DDT with concentration of 61.45 µg/kg, 47.28 µg/kg and 158.97 µg/kg respectively. In this study, the residues of lindane and TDE was not found in sewage sludge sample, possibly due to the concentration of OCP in sewage sludge being lower than the detection limit of the instrument.

The KT samples have more type of OCP residue with the highest concentration of 267.7 µg/kg due to this location being near the industrial and domestic area. Beside that, this sampling point was also near the palm oil estate (~ 50 meter) whereas sources of OCP could originate from pesticides application to the palm oil plants. Based on the "Pesticide Board Malaysia, pp-DDT could still be use for control of the bees and mosquitoes. From the Department of Agriculture Johor, dieldrin and heptachlor could still be used for pesticides to insect, ant and bug control in plantation. Beside that, factory for fertilizer production and chemical processes industry were also sources of OCP found in sludge sample.

In this study LK sample was found to have dieldrin residue due to this location having timber factory and kumai production for pest control, however heptachlor was found in SA sample. Generally, the concentration of OCP for three different sludge sample were far lower compared to the limit from USEPA.

7.7 Summary

Analysis of OCP in sewage sludge using GC-ECD was successfully developed. OCP standard mixture in this study was successfully separated using two different capillary columns; they are HP-5 MS and BPX-5 capillary columns. Soxhlet extraction

method followed by the clean-up technique using silica column was found to be effective in extracting OCP residue in sludge sample.

In this study, several techniques for confirmation of OCP residue were utilized, such as comparison with retention time, co-chromatography and GC-MS techniques. Comparison of retention time and co-chromatography was successful for detection of OCP residue. However, GC-MS was not capable of detecting OCP residue in sample due to the sensitivity of GC-ECD instrument being much better than GC-MS.

Three types of OCPs (dieldrin, heptachlor and p-DDT) residue was found in sewage sample and the sources of OCP were from industrial and domestic activity. Comparison of concentration of OCP in sewage sludge sample with permissible limit from USEPA showed that the sludge samples analyzed were below the maximum level for environmental use.

CHAPTER 8

CONCLUSIONS AND SUGGESTIONS

8.1 Conclusions

This research dealt with the qualitative and quantitative determination of organic contaminants in sewage sludge. Sludge samples were taken from IWK wastewater treatment plants in the Johor area. The criterion of each sampling point was based on its location in industrial areas in Johor and the existence of dying beds in the WTPs. Three major considered were in Johor Bahru, Kluang and Segamat.

The development of new extraction techniques for the analyses of PCBs in sewage sludge was carrier out. Supercritical fluid carbon dioxide and subcritical water extractions were utilised for the removal of PCBs in sewage sludge. These techniques have the added advantages compared to conventional extraction such as reduction of organic solvent use, fast and environment friendly techniques.

All seven PCB congeners under study were successfully separated, with a total time of less than 30 minutes using GC-ECD (on a capillary HP-5MS column) with good linearity and low detection limit in the range of 0.016 to 0.048 μg/mL. For the extract clean-up, silica glass column was found to give high PCB recoveries (84 to 94 %) compared to that using SPE silica (72 to 84 % PCB recovery). Silica column was therefore used in the extract clean-up for the conventional soxhlet extraction

In order to assess the efficiency of the supercritical CO_2 extraction of PCBs, recovery studies of PCBs were conducted using spiked sludge samples. Several parameters such as extraction temperature, extraction pressure, composition of extractant modifier, CO_2 flow-rate, solvent trapping and extraction duration time were optimized for the quantitative recovery of PCBs from blank sludge spiked with PCB standard mixture. The SFE optimized conditions utilised extraction temperature of 60 ^{o}C, extraction pressure of 200 bar, 15 % methanol as co solvent, extraction flow rate at 2.0 mL/min, 30 minute of extraction time and *n*-hexane as the trapping solvent. The recovery studies revealed that SFE afford good PCB recoveries (85 to 99 %) except for PCB 28 with only 55 % recovery.

In this study, an alternative technique using subcritical water was also employed for PCBs extraction. Water, being an "environmental-friendly solvent" has added advantages of being readily available, non-toxic, and cheap. A lab-made SWE extractor was successfully developed for the dynamic extraction of PCBs from sewage sludge samples. For the SWE, the optimized conditions was obtained at extraction temperature of 250 ^{o}C, extraction pressure of 50 bar, 1 min static followed by 10 min dynamic extraction and extract collected in dichloromethane as the trap solvent. Reasonably good PCB recoveries using SWE were obtained (75 to 85 %) except for PCB 28 and PCB 52, yielding only 50 to 59 % recoveries.

Both the new extraction techniques of SFE and SWE gave better recoveries as compared to the conventional soxhlet extraction that gave PCB recoveries ranging from 52 to 75 %. From the extraction methods investigated, is was clear that the SFE technique was better in terms of recovery, speed of analysis and good reproducibility and it was therefore chosen as the method of choice for the analysis of real sludge samples. For the real samples, only four samples were found to contain PCBs; they were from LK, KIS, TI and TY. PCBs in the concentration ranges of 31.2 to 82.0 µg/kg were found in the SFE extracts of the four locations analysed. The PCB levels in sludge samples extracted using soxhlet yielded lower concentration range (20.2 to 60.1 µg/kg) than that using SFE. Sludge samples from LK, KIS, TI and TY contained PCBs due

probably wastewater are originating from medium industrial activities such as battery, electronic, metal and plastic manufacturing in the vicinity of the WTP. Analyte confirmation was carried out using co-injection and GC-MS technique in the SIM mode. All sludge samples were found to contain PCBs well below the EU limit values.

Method development for analysis of PAH in sewage sludge was also carried out using spiked sample and real sample. In this study, extraction of PAH in sewage sludge was utilised using Soxhlet extraction follow by extract clean-up procedure. Two different methods of clean-up was evaluated; they are SPE silica cartridge and silica column. The results showed that both procedures gave quantitative recoveries of PAH, however silica gel column was evidently more efficient than the SPE silica cartridge based on less interference peak chromatogram and more of the PAH analytes could be adsorbed. Hence for this study, the silica gel column was employed for extract clean-up. Limits of detection for five PAH (naphthalene, fluoroanthene, phenanthrene, benzo(a)fluoroanthene and benzo(a)pyrene) were in the range of 5 mg/L to 20 mg/L. Identification of PAH analytes in sewage sludge was carried out using comparison of retention time, co-injection technique, Kovats retention index and GC-MS technique. The results showed that the analytes of naphthalene and phenanthrene were found in three different locations, however other PAHs were not detected in these sludge sample.

Analysis of OCP (lindane, dieldrin, TDE, pp-DDT and heptachlor) in sewage sludge was also successfully developed. The use of GC-ECD Instrument was successful in separating and detecting all OCPs under study. In this study, limits of detection of OCP were in the range 7.8 to 39.0 µg/kg. Extract of OCP in sewage sludge was prepared using Soxhlet extraction followed by clean-up procedure using silica column. Due to low peaks of OCP obtained, identification of OCP analyte in sewage sludge was carried out using comparison of retention time, co-chromatography and GC-MS technique. For the real samples, only three samples were found to contain OCP; they were from LK, SA and KT. LK and SA samples were found to contain lindane and dieldrin; however the KT sample contained dieldrin, pp-DDT and heptachlor. The concentrations of OCP (47 to 159 µg/kg) in sewage sludge were well below the US EPA limit values.

Apart from the determination of organic contaminants content in sludge samples, the samples were also characterized in terms of moisture content, pH of sewage sludge, percentage of total solids and volatile solids, determination of heavy metals (Zn, Cu, Pb, Ni and Cr), and determination of nutrient content in sludge (TKN, P and K). The sludge samples showed high water content 37 to 90 % especially sludge sample taken from oxidation pond and for pH values in this study found are slightly acidic ranging from 4.3 to 6.6. All samples were found to be chemically stable as reflected from the percentage of total solid and volatile solid. From the determination of nutrient in the sewage sludge samples, it is shown that the percentage of the TKN, P and K in sludge were in the range 0.059 to 0.98 %, 0.05 to 0.15 % and 0.12 to 0.16 %, respectively. This sewage sludge has nutrient content much lower than the commercial fertilizer. Heavy metal studies showed that metal content in municipal sewage sludge samples were below the USEPA limit. Low concentration of heavy metals in the sewage sludge samples is amendable for agricultural use since they are valuable contributor of microelements, which have a positive effect on soils and organisms.

6.2 Suggestions for further study

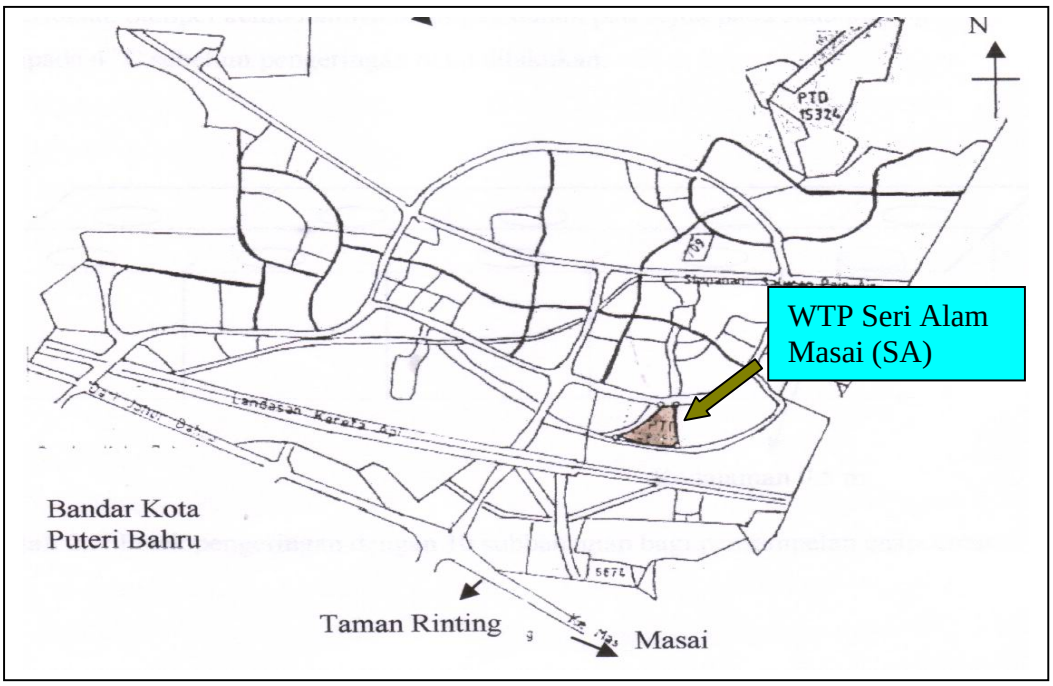
This research marks the beginning for the analysis of organic pollutants in Malaysian sewage sludge. SF-CO_2 and SWE have successful been employed to quant PCBs in sewage sludge with reasonable good recoveries. For future study, PCBs may be analysed along with other organic pollutants such as Linear Alkylbenzene Sulphonated (LAS), Di (2-ethylhexy) phthalate (DEHP), dioxins and Nonylphenole (NP) in sewage sludge due to fact that these contaminants also occur in sewage sludge. The new extraction techniques developed for sludge analysis can also be applied to other matrices such as sludge-amended soil.

Apart from Johor, sludge samples should also be taken from other states in Malaysia. Once the determinations of organic pollutants like PCBs, PAH and OCP have

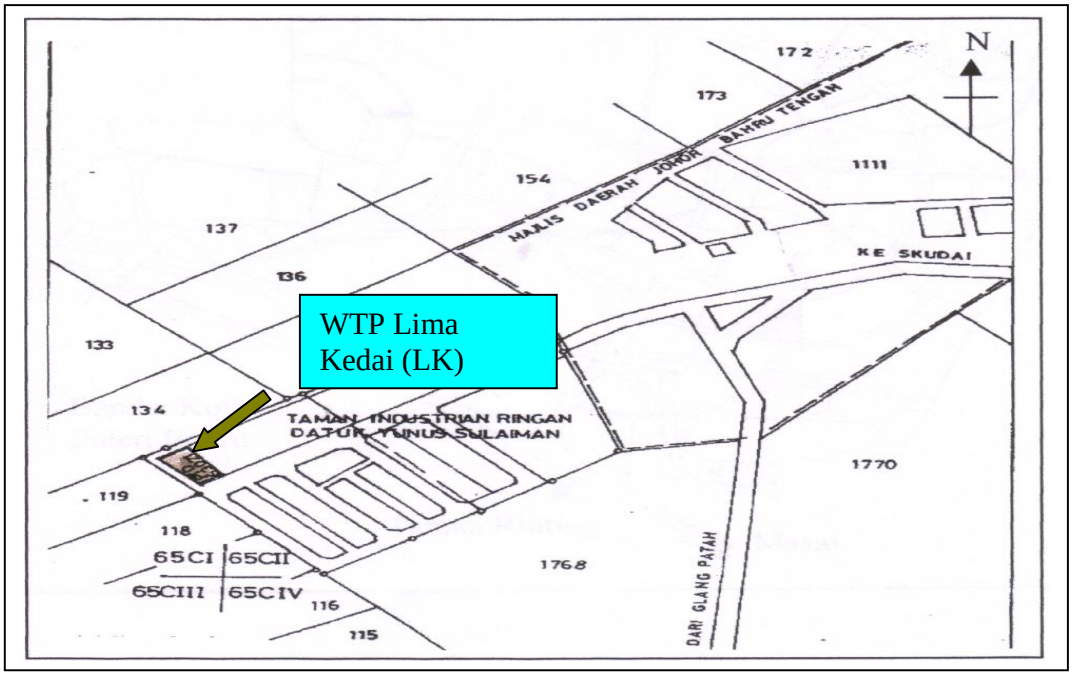
been performed, a regulatory limit value for organic pollutants in Malaysian sludge can be suggested. This is important for sludge to be safely and widely used as fertilizers in Malaysia in the very near future.

APPENDIX A

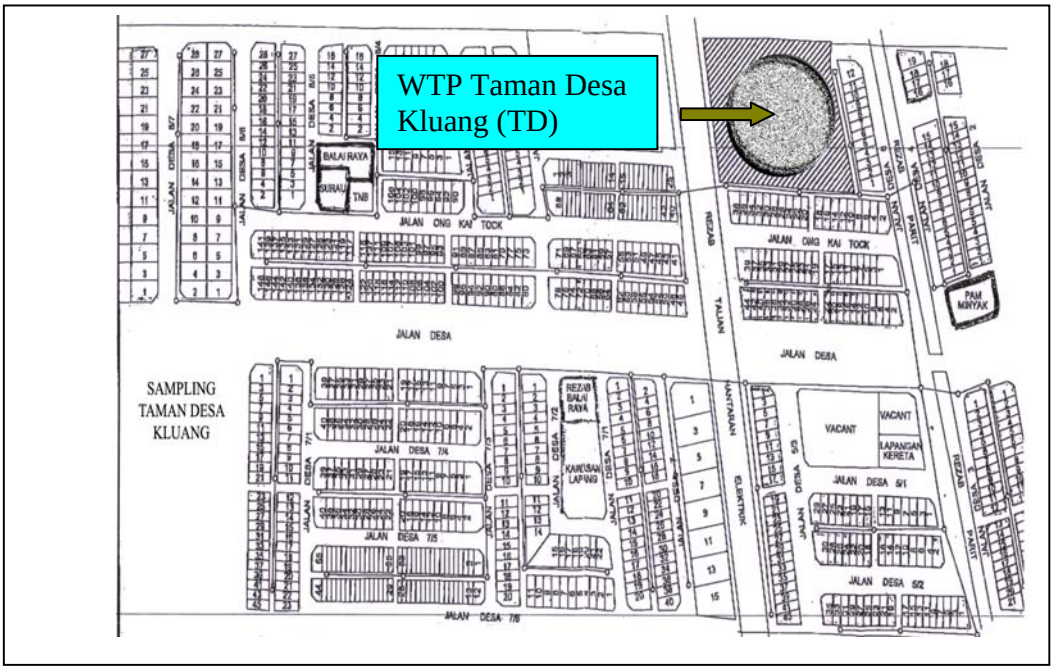
Location of Wastewater Treatment Plant in Industrial/ Housing Area



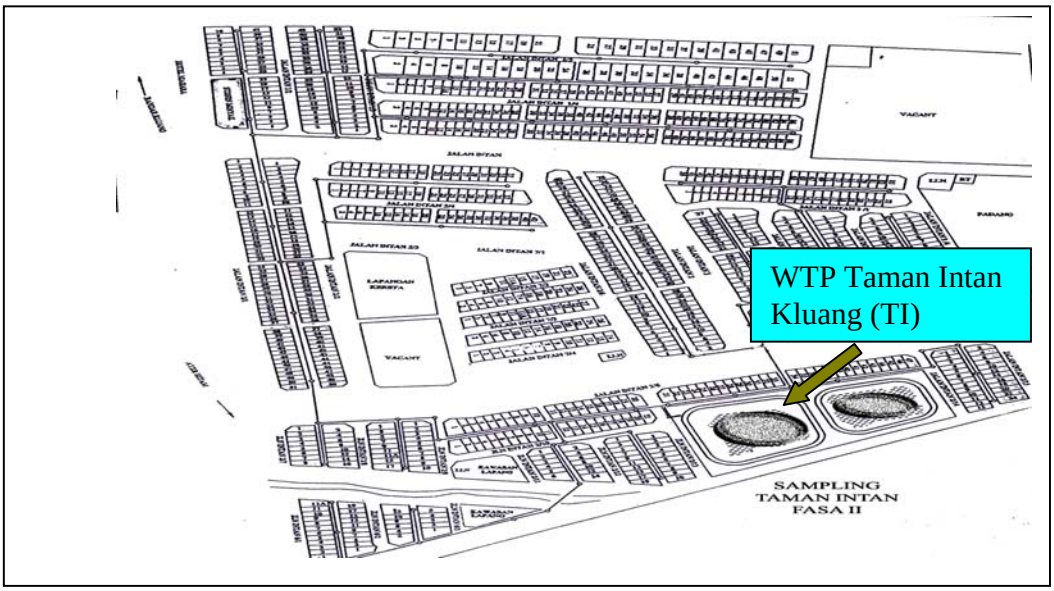
(A) Location of IWK water treatment plant at Seri Alam Masai (SA).



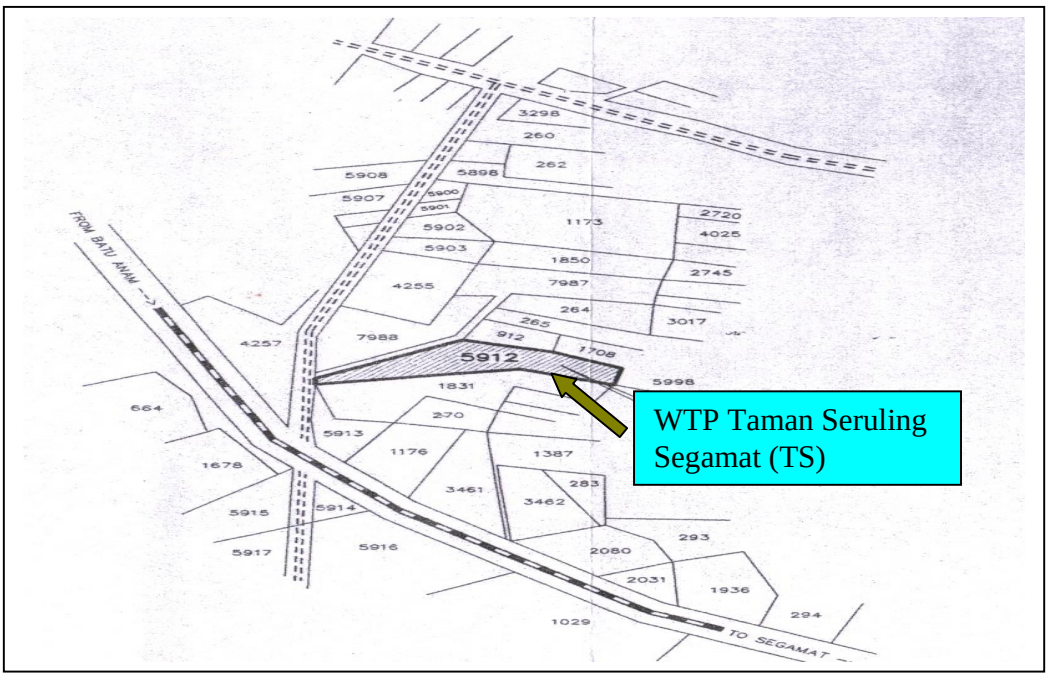
(B) Location of IWK water treatment plant at Lima Kedai Skudai (LK).



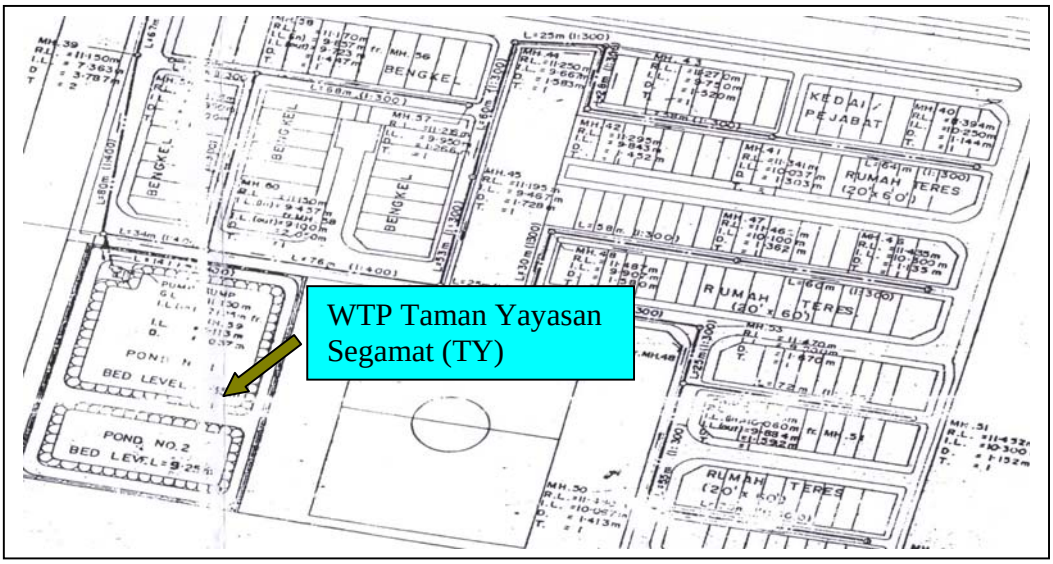
(C) Location of IWK water treatment plant at Taman Desa Kluang (TD).



(D) Location of IWK water treatment plant at Taman Intan Kluang (TI).



(E) Location of IWK water treatment plant at Taman Seruling (TS).



(F) Location of IWK water treatment plant at Taman Yayasan (TY).

APPENDIX B

SFE Recovery Data For PCBs

(A) Effect of extraction temperature on the SFE recovery of the spike sludge using pure CO_2 at 2.0 mL/min with 30 min extraction and extraction pressure of 200 bar.

Temperature (^oC)	Recovery % ±RSD			
PCB	40	50	60	70
28	29.1±2.1	40.2±3.5	46.2±2.4	55.3±3.4
52	35.7±5.1	45.8±3.1	55.5±4.1	60.5±3.7
101	44.3±6.8	60.5±2.1	73.4±6.1	75.2±5.1
118	45.9± 4.7	62.0±5.7	78.3±2.4	74.8±4.1
138	45.1±7.3	62.7±2.2	76.7±6.1	73.9±4.2
153	45.7±6.4	64.7±2.7	79.0±5.4	74.1±5.3
180	47.8±6.1	72.5±2.5	83.6±3.7	79.0±5.7

(B) Effect of extraction pressure on the SFE recovery of the spiked sludge sample using CO_2 at 60 ^oC with 30 min and CO_2 flow rate 2.0 ml/min

Pressure (bar)	Recovery % ±RSD			
PCB	100	200	300	400
28	27.5±2.1	46.2±3.7	57.4±1.5	24.1±5.1
52	45.5±5.1	55.5±8.3	66.5±1.9	25.2±7.7
101	48.5±4.5	73.4±1.3	48.5±6.5	26.1±6.1
118	55.5±3.1	78.3±2.1	42.5±3.2	30.5±4.5
138	64.5±7.8	76.7±5.6	44.5±4.3	30.5±4.6
153	58.5±6.1	79.0±4.1	42.1±2.3	30.5±5.1
180	65.5±6.2	83.6±6.4	40.5±9.6	33.7±5.1

(C) Effect of varying concentration of methanol as modifier on the SFE recoveries of PCBs. SFE conditions: pressure 200 bar, SF flow rate 2.0 mL/min, 60 ^{o}C with 30 minute extraction time.

MeOH %	Recovery % ±RSD			
PCB	0	5	10	15
28	48.1±3.7	49.5±3.5	42.5±3.1	48.77±2.52
52	58.0±8.3	62.0±5.2	90.2±10.2	101.6±9.2
101	65.2±1.3	56.1±4.5	69.3±4.7	83.36±6.21
118	45.5±2.1	65.2±8.2	73.5±1.2	97.01±1.62
138	49.3±5.6	65.0±7.2	75.4±5.4	82.71±4.67
153	68.1±4.1	59.7±6.1	75.8±2.7	77.77±5.87
180	81.1±6.4	67.2±6.7	89.0±2.9	92.82±3.85

(D) Effect of varying flow rate on the SFE recoveries PCBs. SFE conditions: pressure 200 bar, SF-CO_2 with 15 % methanol as modifier and 60 ^{o}C with 30 minute extraction time.

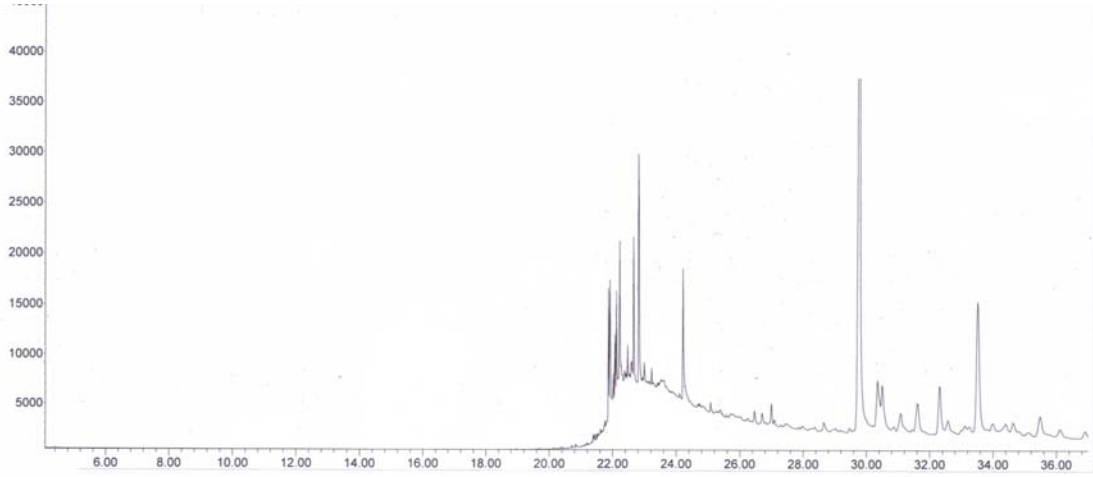
Flow rate (mL/min)	Recovery % ±RSD			
PCB	0	5	10	15
28	12.10±9.1	19.2±5.2	48.7±5.2	29.9± 8.5
52	19.21±5.1	22.4±8.3	101.6±8.2	86.2±7.1
101	23.1±4.5	23.2±2.1	83.3±6.3	52.5±8.1
118	4.2±2.3	27.1±3.1	97.0±2.8	57.2±4.7
138	27.3±7.1	27.5±5.1	82.7±5.3	55.3±4.3
153	29.3±6.1	27.9±2.5	77.7±2.3	46.9±5.2
180	29.2±6.3	26.1±2.7	92.8±2.2	69.4±5.4

(E) Effect of different solvent trapping on the SFE recoveries of PCB congener. SFE conditions: pressure 200 bar, temperature 60 ^{o}C, 15 % methanol as modifier and with 30 minute extraction time

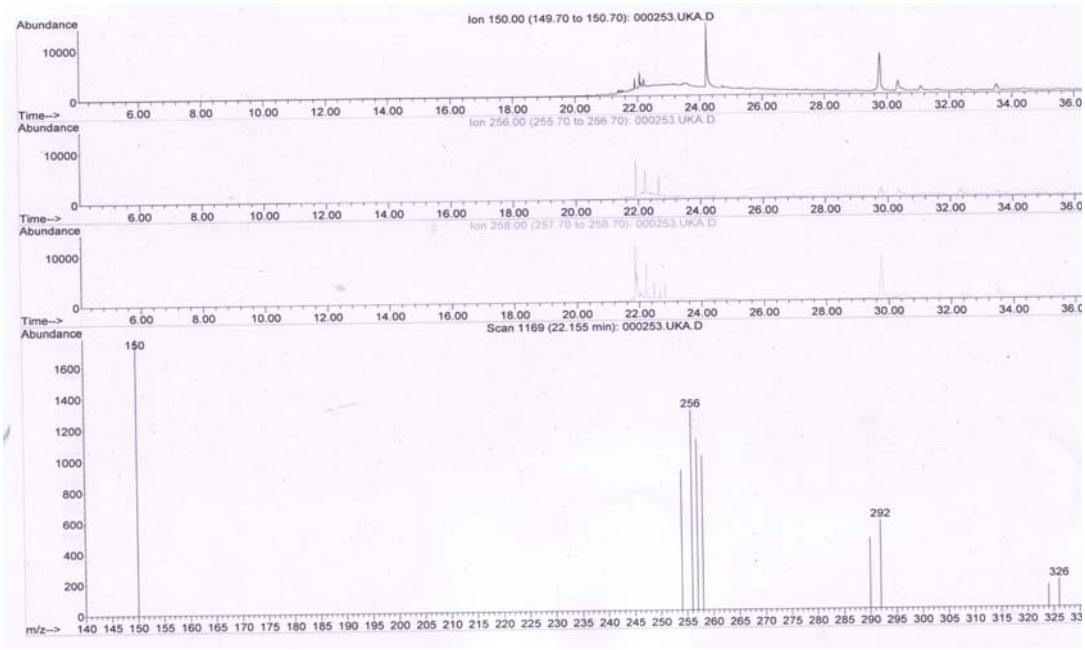
Solvent Trapping	Recovery %±RSD		
PCB	Methanol	*Iso*-Octane	*n*-Hexane
28	48.7±9.1	69.13±3.1	55.2±2.5
52	101.6±9.1	102.21±8.1	91.9±9.2
101	83.3±4.1	66.37±4.3	91.0±6.2
118	97.0±1.2	101.1±9.0	99.2±1.6
138	82.7±7.2	74.90±1.8	85.1±4.6
153	77.7±6.7	74.4±2.7	91.4±5.8
180	92.8±5.6	84.3±2.2	96.3±3.8

APPENDIX C

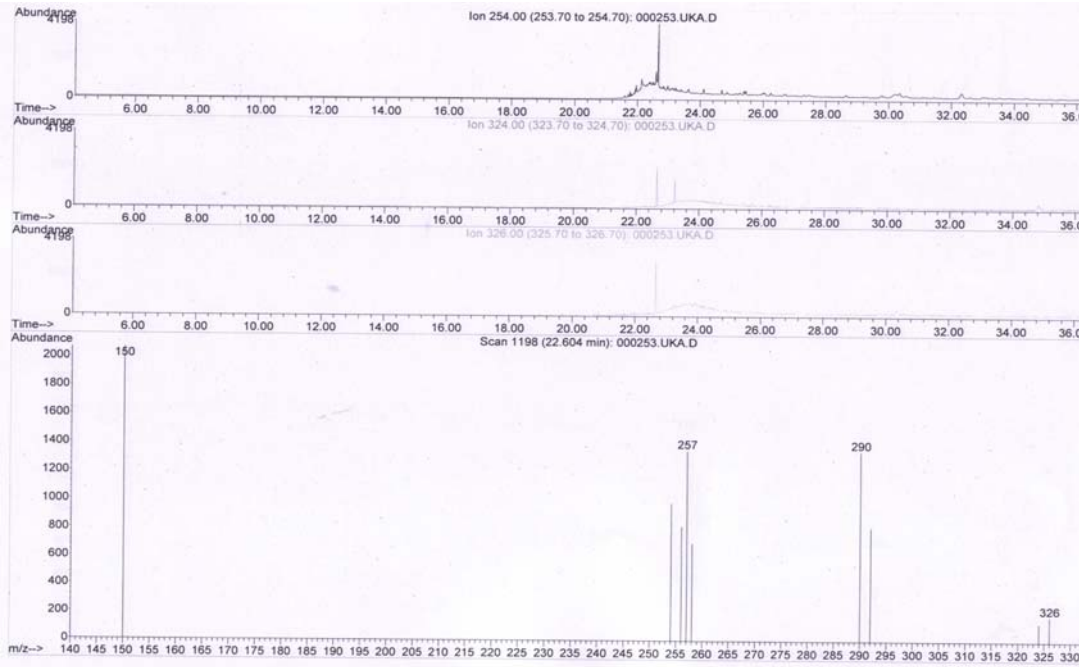
SIM Spectrum and extract ion chromatogram for PCB peak confirmation



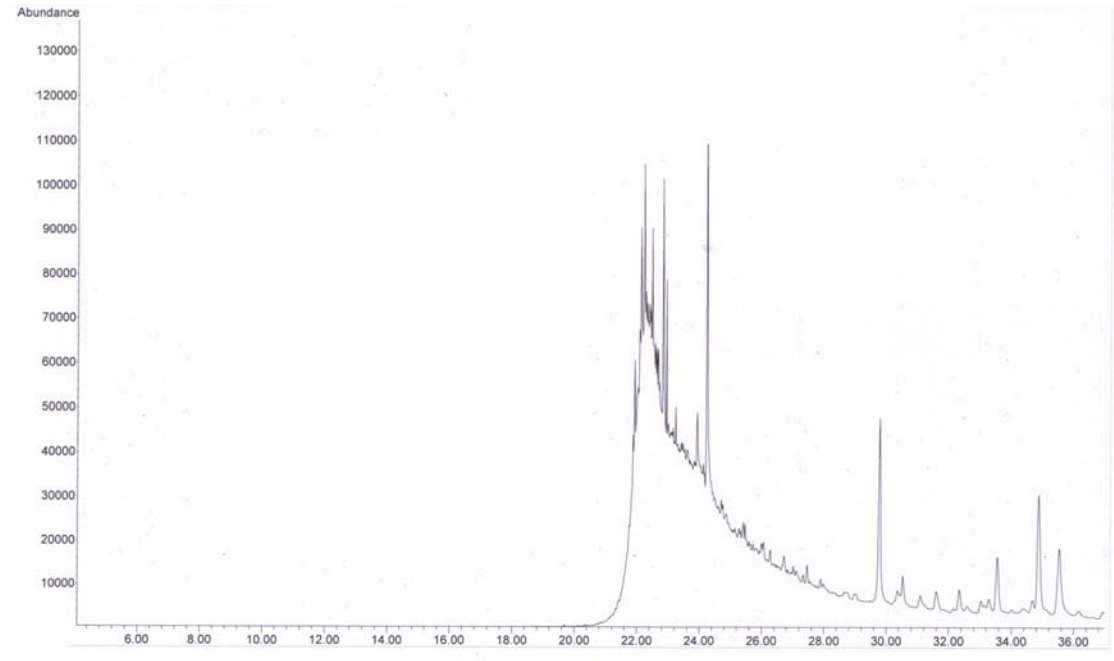
(a) Total ion chromatogram for sample KIS using GC-MS-SIM mode.



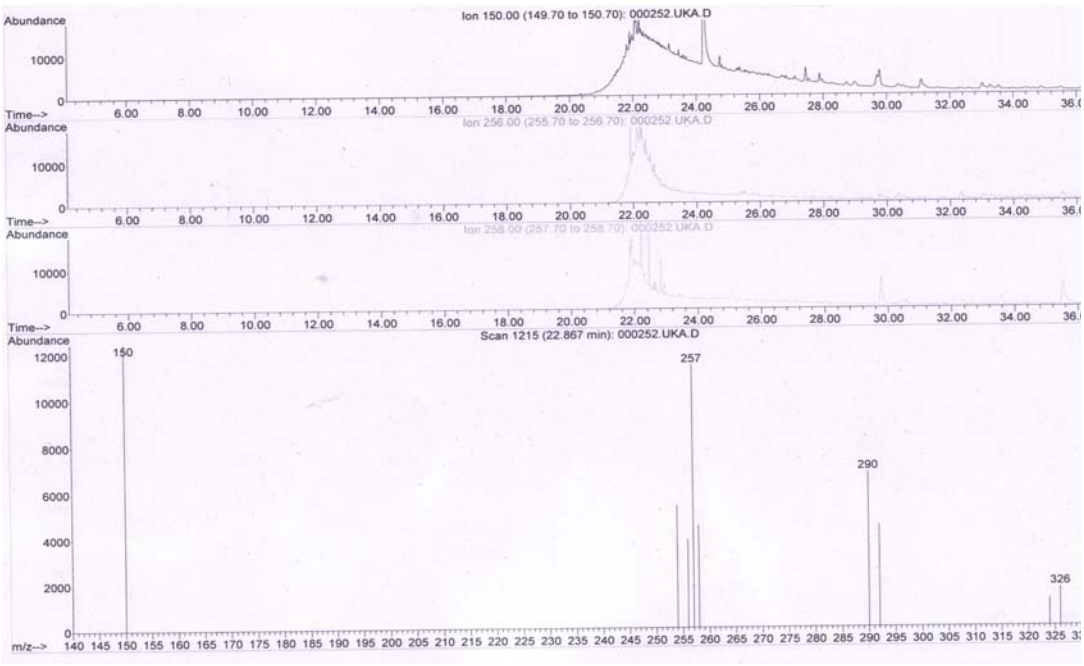
(b) Extract ion chromatogram for sample KIS to confirm PCB 28 (m/z 150, 256 and 258).



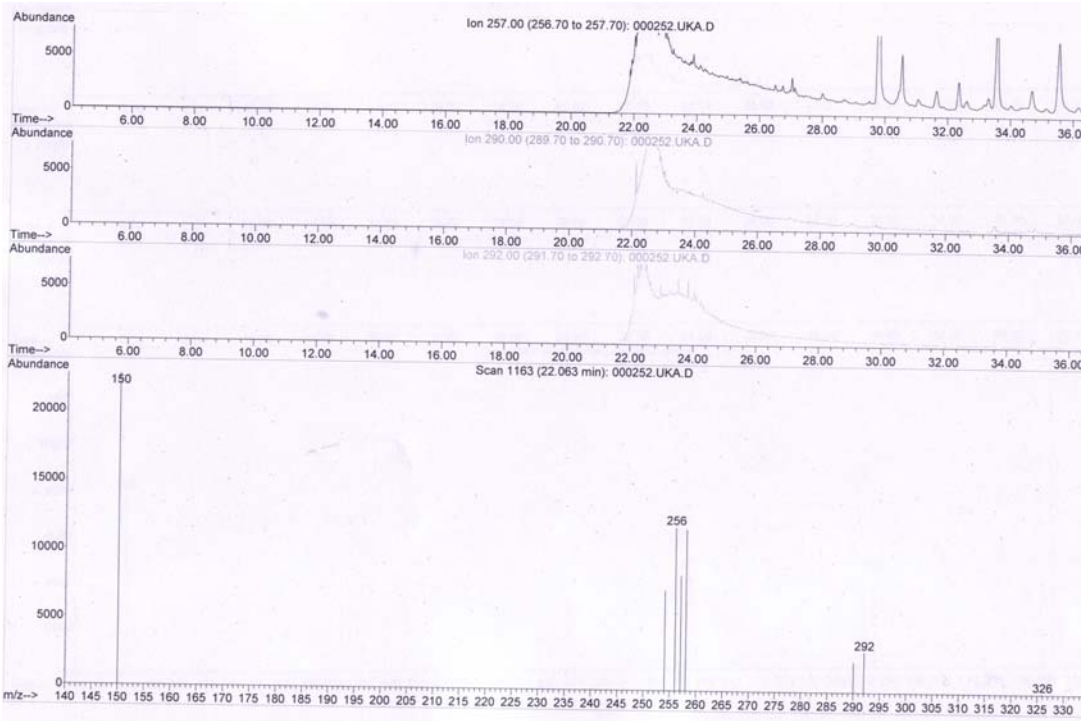
(c) Extract ion chromatogram for sample KIS to confirm PCB 101 (m/z 254, 324 and 326).



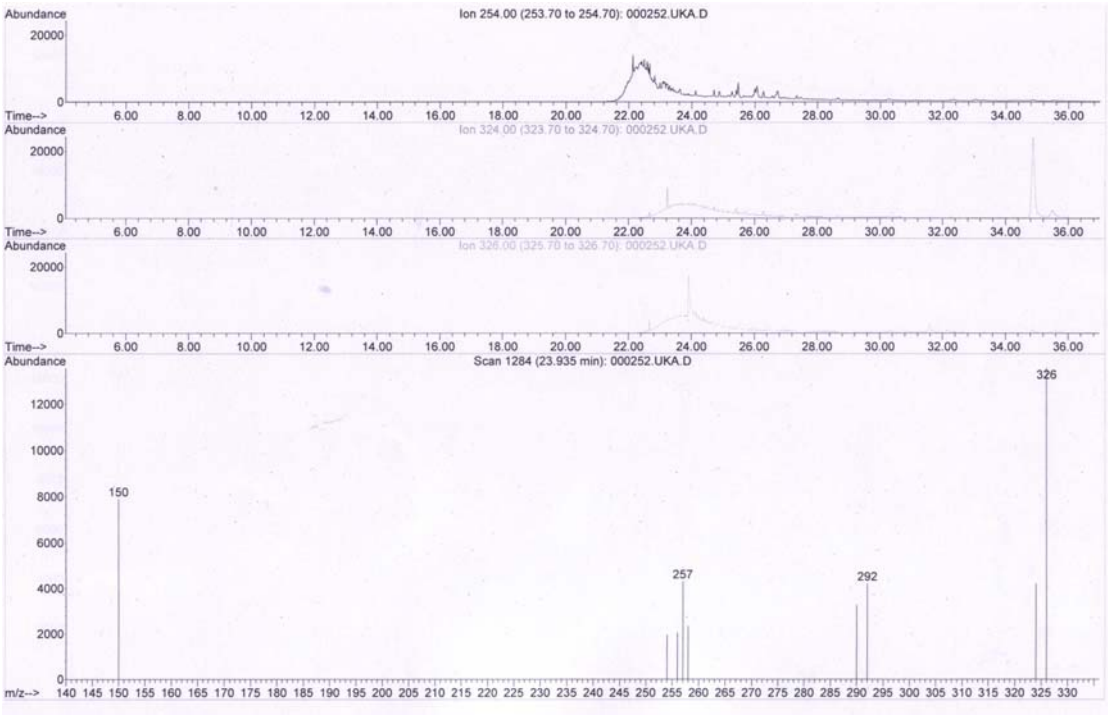
(d) Total ion chromatogram for sample LK using GC-MS-SIM mode



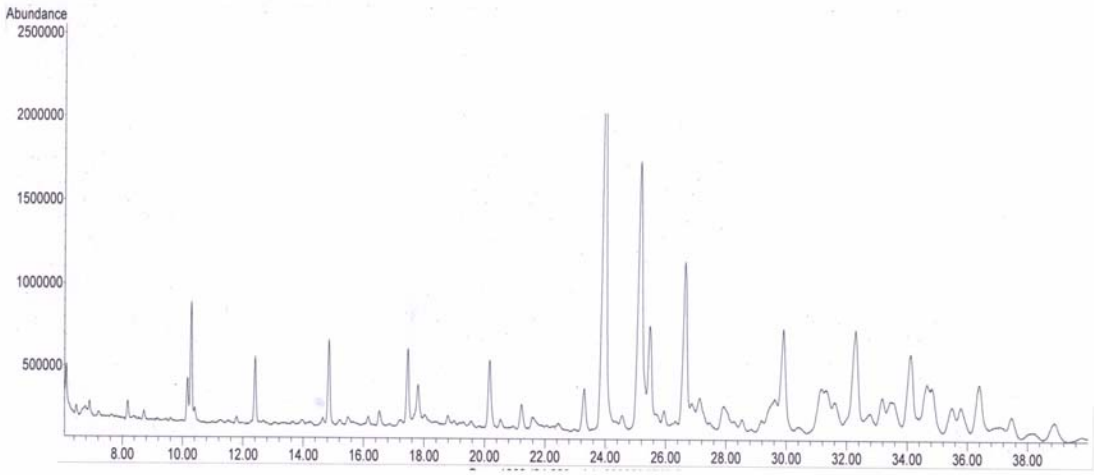
(e) Extract ion chromatogram for sample LK to confirm PCB 28 (m/z 150, 256 and 258)



(f) Extract ion chromatogram chromatogram for sample LK to confirm PCB 52 (m/z 257,290 and 292).



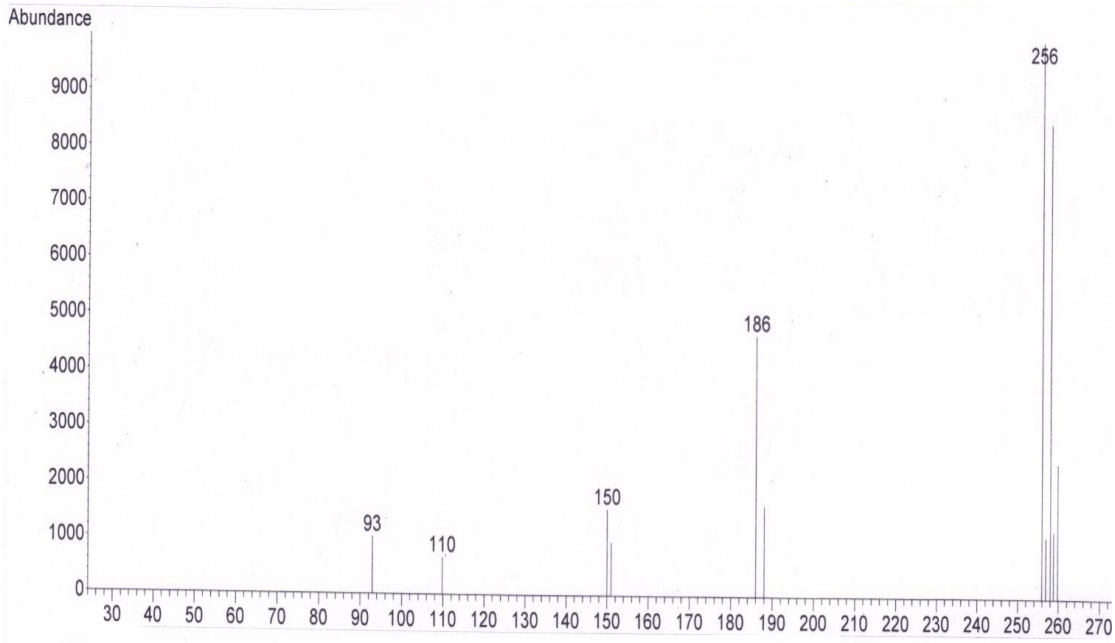
(g) Extract ion chromatogram for sample LK to confirm PCB 28 (m/z 254, 324 and 326).



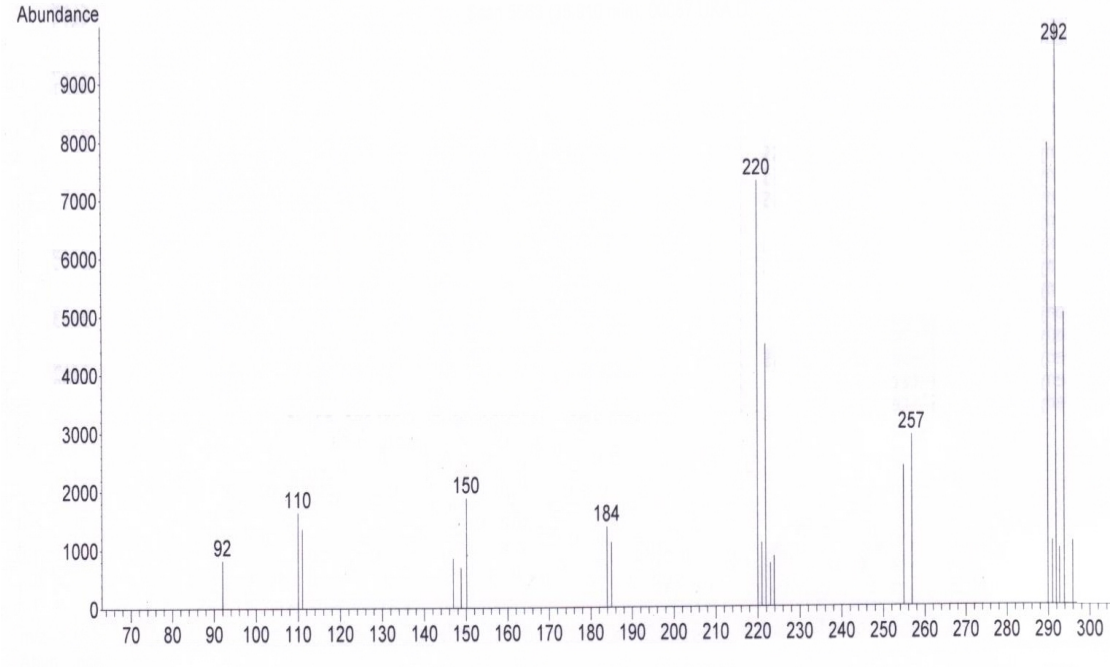
(f) Total ion chromatogram for sample TI using GC-MS-SIM mode

APPENDIX D

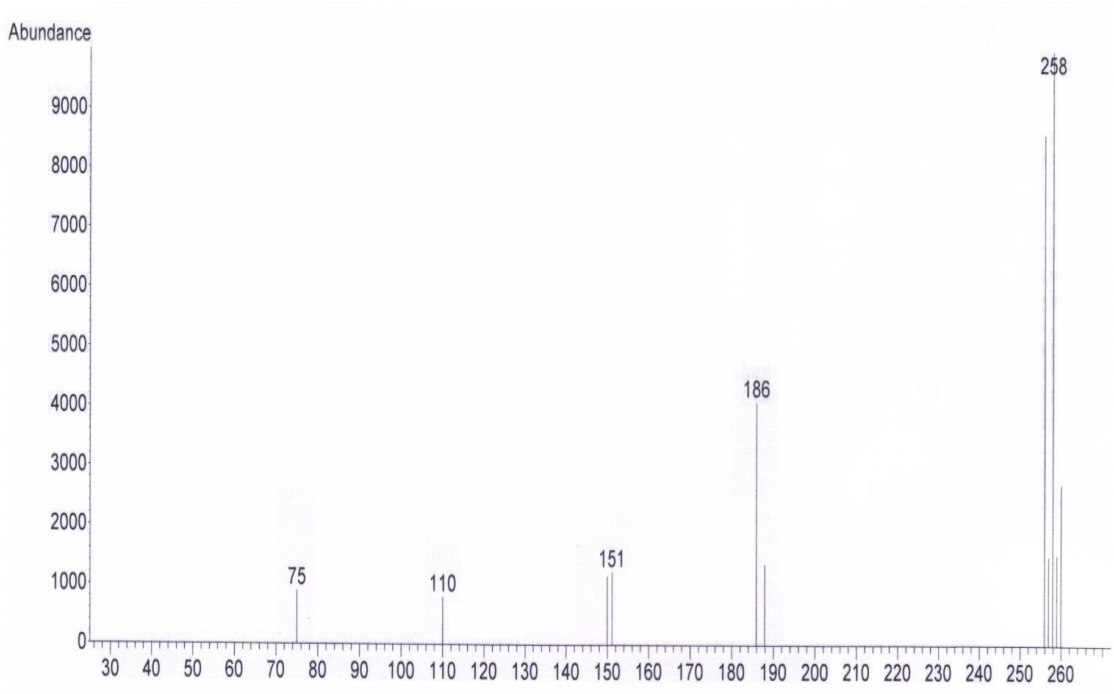
Mass Spectrum for PCBs Standard



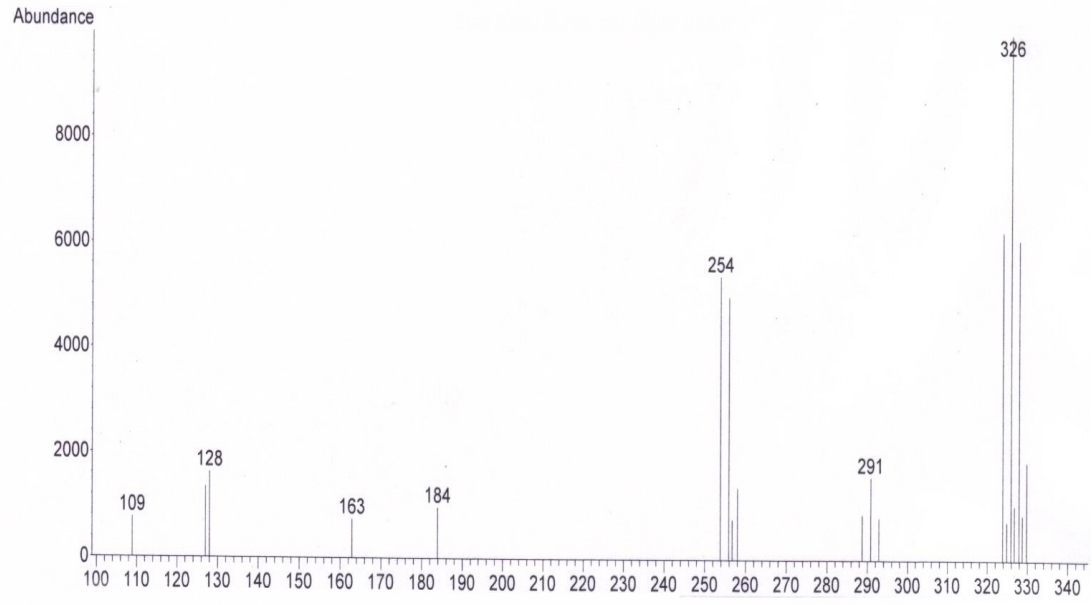
(A) Mass spectrum for PCB 28



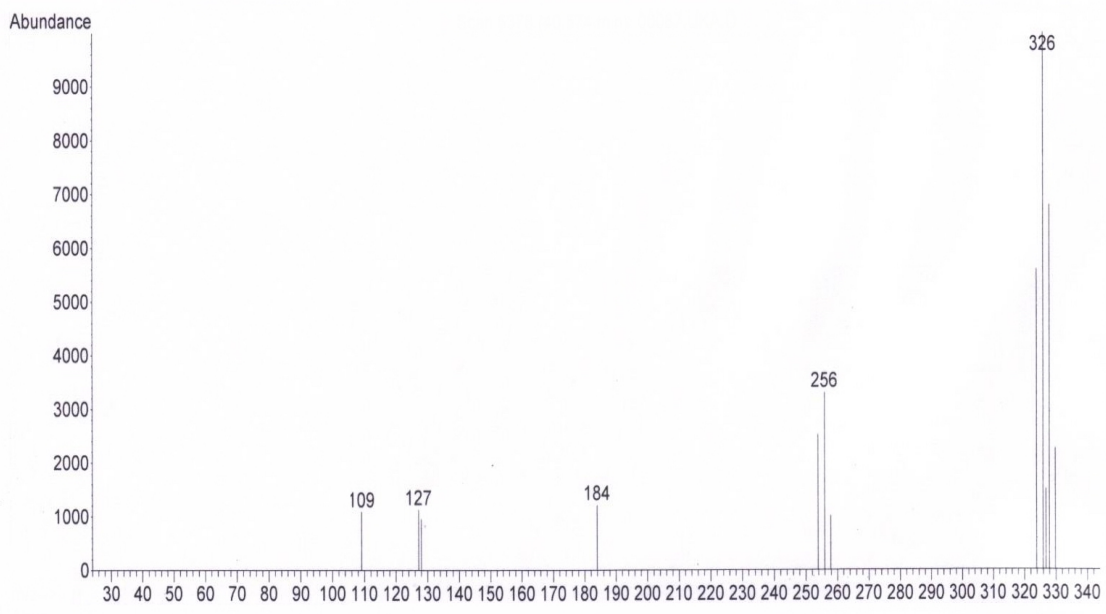
(B)Mass spectrum for PCB 52



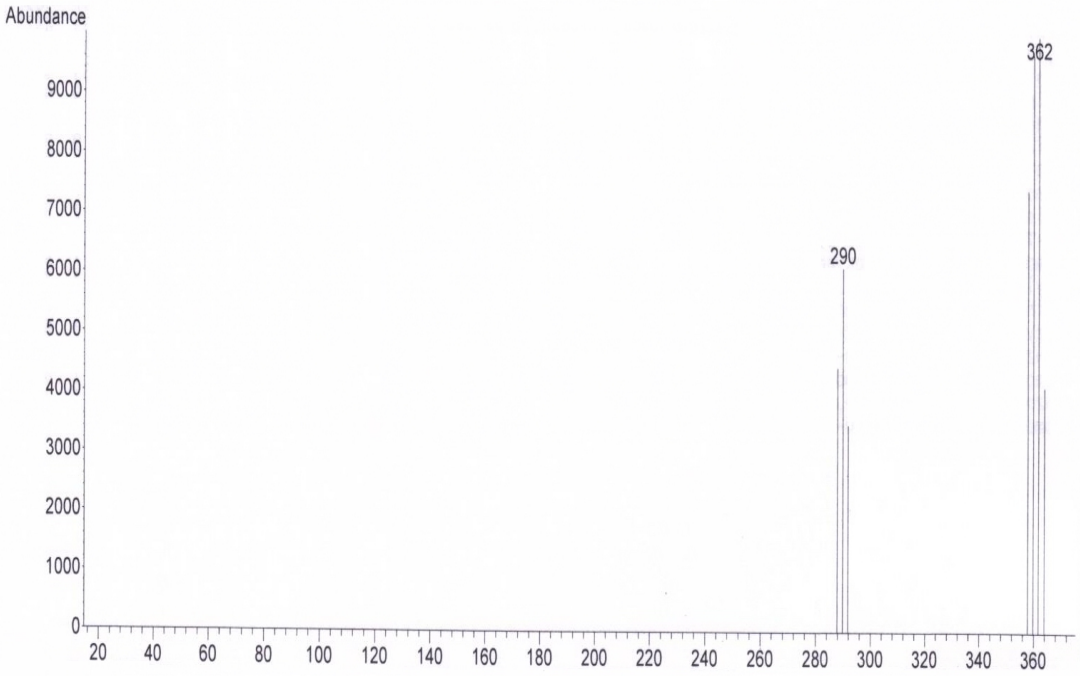
(C) Mass spectrum for PCB 35



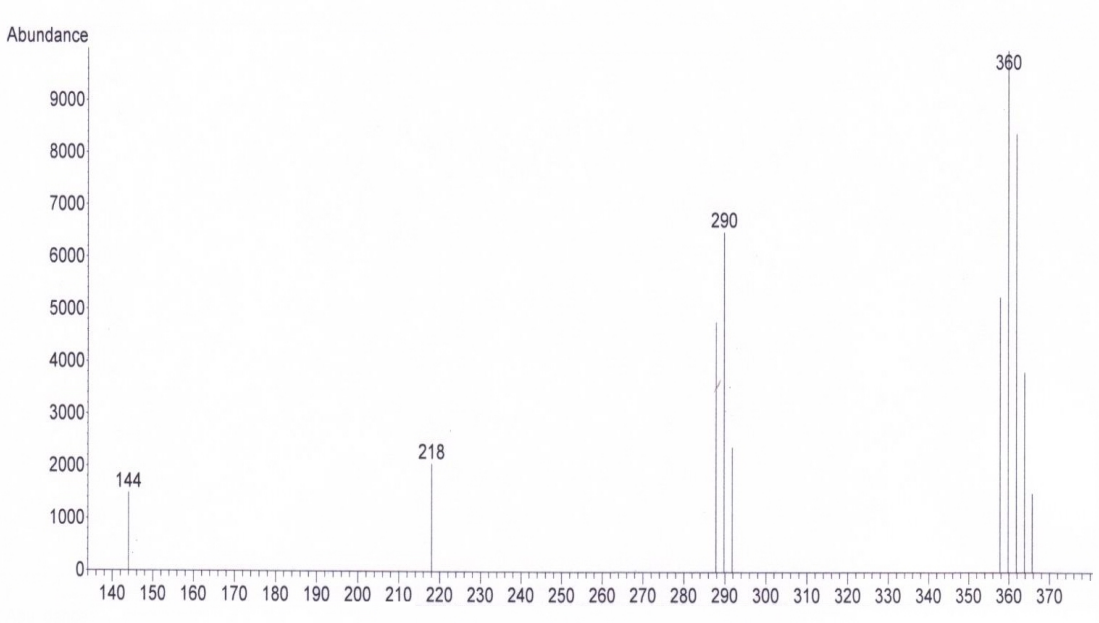
(D) Mass spectrum for PCB 101



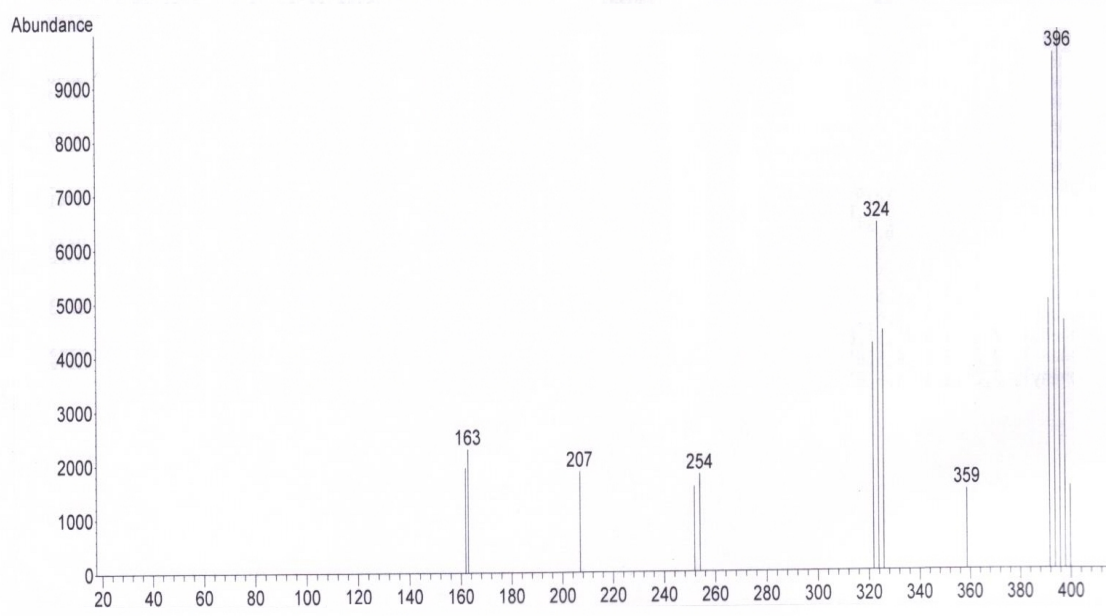
(E) Mass spectrum for PCB 118



(F) Mass spectrum for PCB 153



(G) Mass spectrum for PCB 138



(H) Mass spectrum for PCB 180

APPENDIX E

Presentation and Publications

Parts of the research study have been presented at the following symposia;

1. Chong Huey Woon (2002). Analisis Pencemar Organik Hidrokarbon Aromatik Polisiklik (PAH) dan Pestisid Organoklorin (OCP) dalam Sampel Enapcemar. MSc. Thesis Universiti Teknologi Malaysia, Skudai. Johor.

2. Umi K. Ahmad and Ahmad Zamani Ab Halim (2004). Determination of Polychlorinated Biphenyl in Sewage Sludge and Oil Palm. 17th Symposium of the Malaysian Analytical Chemistry, (SKAM 17). Swiss Garden Hotel. Kuantan. Pahang.

3. Umi K. Ahmad and Ahmad Zamani Ab Halim (2005). Development of a Subcritical Water Extraction for the Quantitative Determination of Polychlorinated Biphenyls in Sewage Sludge. 18th Symposium of the Malaysian Analytical Chemistry, (SKAM 18). Hyatt Hotel. Johor Bahru.

4. Umi K. Ahmad and Ahmad Zamani Ab Halim (2006). Supercritical Fluid Carbon Dioxide for the Extraction of Polychlorinated Biphenyls in Sewage Sludge. Regional Postgraduate Conference on Engineering and Science 2006. Universiti Teknologi Malaysia, Skudai Johor.

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